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**(54) MORPHOGEN-INDUCED DENTINE REGENERATION**

**MORPHOGENINDUZIERTE REGENERIERUNG DER DENTIN**

**REGENERATION DE LA DENTINE INDUITE PAR UN MORPHOGENE**

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- **MOL. PATHOG. PERIODONTAL DIS.** (1994), 427-37. **EDITOR(S): GENCO, ROBERT.** **PUBLISHER: AM. SOC. MICROBIOL., WASHINGTON, D. C. CODEN: 61LAA2, 1994, XP000576244 RUTHERFORD, BRUCE ET AL:** "Role of osteogenic ( bone morphogenetic ) protein and platelet-derived growth factor in periodontal wound healing"
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**EP 0 812 207 B1**

**Description****Field of the Invention**

**[0001]** The present invention relates generally to the dental and biomedical arts. In certain embodiments, the invention more particularly relates to methods and compositions for stimulating mammalian odontoblasts and inducing morphogenesis of mammalian dentine.

**Background of the Invention**

**[0002]** In mammals, periodontal disease, such gingivitis, can arise from the weakening of periodontal tissue by infectious agents (e.g., buccal microorganisms), nutritional deficiency (e.g. scurvy), or neoplastic disease (e.g., leukemia and lymphoma). Periodontal diseases often are characterized by inflammation, bleeding, tissue recession and/or ulceration. If not properly treated, periodontal diseases can contribute to tooth loss. For example, gingival lesions can arise where bacterial plaque adheres to the tooth/gingiva interface and provokes local inflammation and/or recession of the gingiva. In the early stages, gingivitis is associated with tooth sensitivity to perception of pressure and/or temperature. For example, afflicted teeth may ache upon contact with cold or hot stimuli. If untreated, this progresses to severe continual throbbing pain, ultimately associated with infection of the tooth pulp tissue, periodontal ligament, or alveolar bone of the tooth socket. More severe complications, e.g., endocarditis, can arise where untreated lesions provide buccal microorganisms with a portal of entry into the afflicted individual's bloodstream. Harrison's Principles of Internal Medicine, 12th edition, 1991 (Wilson et al., eds.), pp. 242-243. Current treatments include professional cleaning to remove plaque and tartar, use of oral antiseptics, local and/or systemic antibiotic therapies, and/or surgical procedures to remove periodontal pockets formed from periodontal tissue lesions and necrosis. Gingivitis thus is treated by debridement of lesioned gingiva and the affected tooth or tooth root surface adjoining the lesion site. Treated gingival lesions heal through the formation of scar tissue at the lesion site. Where tooth loss is imminent or has already occurred as a result of periodontal disease, a prosthetic tooth or removable bridge is substituted for the natural tooth.

**[0003]** Dental caries also is generally attributable to the weakening of tooth tissue by infectious agents or nutritional causes. A cavity, or carious lesion, often involves colonization and degradation of mineralized tooth tissue (e.g., enamel or dentine) by buccal microorganisms. If untreated, the lesion site expands and can weaken, permeating the mineralized tooth wall and placing the tooth pulp tissue at risk of infection. Thus, an untreated carious lesion site also can provide buccal microorganisms with a portal of entry into the bloodstream. Conventional treatments for dental caries include ablation of lesioned dentine to expose a fresh surface of unaffected residual dentine, followed by sealing and restoration with an inert material suitable for dental use, e.g., silver amalgam, composite plastic, gold or porcelain. If infection has spread to the pulp tissue, it becomes necessary to extract the tooth or remove the contents of the pulp chamber and root canals prior to sealing and reconstruction with inert materials. Both approaches require the construction of permanent dental prostheses, such as bridges or crowns, which can become brittle over time.

**[0004]** Previously disclosed are methods and compositions capable of inducing periodontal tissue morphogenesis and dentinogenesis in a mammal, including a therapeutically effective concentration of a morphogen (U.S.S.N. 08/155,343 (published as WO94/06399)). Yet, needs remain for improved treatment of dental caries and periodontal disease, including gingivitis. Particular needs remain for improved treatment methods and compositions which mitigate loss of teeth and associated tissue, including dentine, gingiva and pulp tissue. Still more particular needs remain for improved methods and compositions which allow for the regenerative healing of functional dental tissues following resection of carious or periodontal lesions, including dentine tissue, pulp, cementum, periodontal ligament, gingiva and the like.

**Summary of the Invention**

**[0005]** It is an object of this invention to provide means for inhibiting loss of dental tissue in mammals, as well as means for inducing regeneration thereof. It is an object of the present invention to provide means for stimulating proliferation and differentiation of odontoblasts in mammals, particularly primates. It also is an object of the present invention to provide means for stimulating expression of the odontoblast phenotype, including production of mineralized dentine matrix, by mammalian tooth pulp tissue, including primate tooth pulp tissue such as human tooth pulp tissue. Another object is to provide means for inhibiting the periodontal tissue damage and tooth loss associated with periodontal and other gum diseases, including gingivitis. Additional objects include providing means for desensitizing teeth to perception of pressure or temperature, as well as for sealing a tooth cavity by inducing formation of reparative dentine tissue. These and other objects, along with advantages and features of the invention disclosed herein, will be apparent from the description, drawings and claims that follow.

**[0006]** The invention is defined in the claims appended hereto. The invention provides methods and compositions

for inhibiting periodontal and tooth tissue (collectively, dental tissue) loss in a mammal, particularly a human, including regenerating damaged tissue and/or inhibiting additional damage thereto. The methods and compositions of this invention can be used to prevent and/or inhibit tooth loss associated with gingivitis and other periodontal diseases. The present methods and compositions also can be used to desensitize teeth to perception of pressure and/or temperature, and pain associated, therewith in dental caries and gingivitis. The invention further provides methods and compositions for stimulating morphogenesis of mammalian dentine, including stimulating proliferation and differentiation of odontoblasts. In particular, the invention provides methods and compositions for stimulating expression of the odontoblast phenotype, including production of dentine matrix, by tooth pulp tissue in mammals, including primates. The present invention can be used to seal a cavity in a mammalian tooth by inducing the formation of reparative dentine. Thus, the invention reduces the need for tooth extraction or root canal therapy as treatments for dental caries or other dental damage in which pulp tissue is placed at risk.

**[0007]** The methods and compositions of this invention capitalize on the discovery that certain proteins of eukaryotic origin, defined herein as morphogens, induce morphogenesis of functional cells, tissues and organs in higher eukaryotes, particularly mammals, including humans. That is, morphogens induce or reinduce the fully integrated developmental cascade of cellular and molecular morphogenetic events that culminate in the formation of fully differentiated, functional tissue of a type appropriate to the context or local environment in which morphogenesis is induced, including any vascularization, connective tissue formation, innervation and the like characteristic of the naturally-occurring tissue. Morphogenesis therefore differs significantly from simple reparative healing processes in which scar tissue (e.g., fibrous connective tissue) is formed and fills a lesion or other defect in differentiated, functional tissue. Further, morphogenesis occurs in a "permissive environment" by which is meant a local environment that does not stifle or suppress morphogenesis (e.g., regeneration or regenerative healing). Permissive environments exist, e.g., in embryonic tissue or in wounded or diseased tissue, including tissue subjected to surgical intervention. Often, a permissive environment comprises a suitable matrix or substratum to which cells undergoing differentiation can anchor. Other components of a permissive environment typically include signals, e.g., cell surface markers or extracellular matrix components, that direct the tissue specificity of differentiation.

**[0008]** Generally, morphogens are dimeric proteins that induce morphogenesis of one or more eukaryotic (e.g., mammalian) cells, tissues or organs. Of particular interest herein are morphogens that induce morphogenesis at least of mammalian dentine, including formation of reparative dentine at or apposite to a dental or periodontal lesion site in a mammalian tooth. Morphogens comprise a pair of polypeptides that, when folded, adopt a configuration sufficient for the resulting dimeric protein to elicit morphogenetic responses in cells and tissues displaying receptors specific for said morphogen. That is, morphogens generally induce all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. "Progenitor" cells are uncommitted cells that are competent to differentiate into one or more specific types of differentiated cells, depending on their genomic repertoire and the tissue specificity of the permissive environment in which morphogenesis is induced. Morphogens further can delay or mitigate the onset of senescence- or quiescence-associated loss of phenotype and/or tissue function. Morphogens still further can stimulate phenotypic expression of differentiated cells, including expression of metabolic and/or functional, e.g., secretory, properties thereof. In addition, morphogens can induce redifferentiation of committed cells under appropriate environmental conditions. As noted above, morphogens that induce proliferation and differentiation at least of mammalian odontoblasts, and/or support the growth, maintenance and functional properties of mammalian odontoblasts, including the formation of dentine matrix, are of particular interest herein. For purposes of the present invention, an "odontoblast" is any differentiated cell occurring or arising in mammalian tooth pulp tissue, that is competent to produce dentine matrix.

**[0009]** In preferred embodiments, the pair of morphogen polypeptides have amino acid sequences each comprising a sequence that shares a defined relationship with an amino acid sequence of a reference morphogen. Herein, preferred morphogen polypeptides share a defined relationship with a sequence present in morphogenically active human OP-1, Seq. ID No. 4. However, any one or more of the naturally occurring or biosynthetic sequences disclosed herein similarly could be used as a reference sequence. Preferred morphogen polypeptides share a defined relationship with at least the C-terminal six cysteine domain of human OP-1, residues 43-139 of Seq. ID No. 4. Preferably, morphogen polypeptides share a defined relationship with at least the C-terminal seven cysteine domain of human OP-1, residues 38-139 of Seq. ID No. 4. That is, preferred morphogen polypeptides in a dimeric protein with morphogenic activity each comprise a sequence that corresponds to a reference sequence or is functionally equivalent thereto.

**[0010]** Functionally equivalent sequences include functionally equivalent arrangements of cysteine residues disposed within the reference sequence, including amino acid insertions or deletions which alter the linear arrangement of these cysteines, but do not materially impair their relationship in the folded structure of the dimeric morphogen protein, including their ability to form such intra- or inter-chain disulfide bonds as may be necessary for morphogenic activity. Functionally equivalent sequences further include those wherein one or more amino acid residues differs from the corresponding residue of a reference morphogen sequence, e.g., the C-terminal seven cysteine domain (also

referred to herein as the conserved seven cysteine skeleton) of human OP-1, provided that this difference does not destroy morphogenic activity. Accordingly, conservative substitutions of corresponding amino acids in the reference sequence are preferred. Amino acid residues that are "conservative substitutions" for corresponding residues in a reference sequence are those that are physically or functionally similar to the corresponding reference residues, e.g., that have similar size, shape, electric charge, chemical properties including the ability to form covalent or hydrogen bonds, or the like. Particularly preferred conservative substitutions are those fulfilling the criteria defined for an "accepted point mutation" in Dayhoff et al. (1978), 5 Atlas of Protein Sequence and Structure, Suppl. 3, ch. 22 (pp. 354-352), Natl. Biomed. Res. Found., Washington, D.C. 20007.

**[0011]** In certain embodiments, a polypeptide suspected of being functionally equivalent to a reference morphogen polypeptide is aligned therewith using the method of Needleman et al. (1970), 48 J. Mol. Biol. 443-453, implemented conveniently by computer programs such as the Align program (DNASTar, Inc). As noted above, internal gaps and amino acid insertions in the candidate sequence are ignored for purposes of calculating the defined relationship, conventionally expressed as a level of amino acid sequence homology or identity, between the candidate and reference sequences. "Amino acid sequence homology" is understood herein to mean amino acid sequence similarity. Homologous sequences share identical or similar amino acid residues, where similar residues are conservative substitutions for or "allowed point mutations" of corresponding amino acid residues in an aligned reference sequence. Thus, a candidate polypeptide sequence that shares 70% amino acid homology with a reference sequence is one in which any 70% of the aligned residues are either identical to or are conservative substitutions of the corresponding residues in a reference sequence.

**[0012]** Of particular interest herein are morphogens, which, when provided to the tooth and/or jawbone surfaces in a mammalian tooth socket, induce periodontal tissue formation where periodontal tissue has been lost or damaged. Of still more particular interest herein are morphogens which, when applied to a tooth surface, such as a dentinal surface, induce morphogenesis of new or reparative dentine. Such morphogens can be used to seal a tooth cavity or to desensitize a tooth to perception of pressure and/or temperature.

**[0013]** The present invention alternatively can be practiced with methods and compositions comprising a morphogen stimulating agent in lieu of a morphogen. A "morphogen stimulating agent" is a compound that stimulates *in vivo* production, e.g., expression, of a therapeutically effective concentration of an endogenous morphogen in the body of the mammal sufficient to regenerate damaged dental tissue and/or to inhibit additional damage thereto. Such compounds are understood to include substances which, when administered to a mammal, act on cells of tissue(s) or organ(s) that normally are competent to produce and/or secrete a morphogen encoded within the genome of the mammal, and which cause the endogenous level of the morphogen in the mammal's body to be altered. Endogenous or administered morphogens can act as endocrine, paracrine or autocrine factors. That is, endogenous morphogens can be synthesized by the cells in which morphogenetic responses are induced, by neighboring cells, or by cells of a distant tissue, in which circumstances the secreted endogenous morphogen is transported to the site of morphogenesis e.g., by the individual's bloodstream. In preferred embodiments, the agent stimulates expression and/or secretion of an endogenous morphogen so as to increase amounts thereof in dental tissues, such as alveolar bone, periodontium, cementum, dentine or pulp tissue cells.

**[0014]** In certain preferred aspects of the present invention, the morphogens described herein can induce regeneration of damaged or lost dentine tissue in a mammalian tooth. The morphogen can be provided topically or otherwise administered to the tooth tissue. For example, the morphogen can be dispersed in a biocompatible, porous carrier material that then is provided topically to the damaged dentine tissue. A useful carrier can be formulated from suitable organ specific tissue, e.g., bone or dentine, by demineralizing and guanidine-extracting the tissue to create an acellular matrix as described in U.S.S.Nos. 07/971,091 (published as WO94/10203), 08/155,343 (published as WO94/06399) and 08/174,605 (published as WO94/06420). Synthetic materials also can be used. In some embodiments, the existing tooth tissue provides a suitable matrix. If a formulated matrix or carrier is used, it should be a biocompatible, suitably modified acellular matrix having dimensions such that it allows the differentiation and proliferation of migratory progenitor cells, and contributes to a morphogenically permissive environment. Preferably, the matrix allows cellular attachment and is biodegradable or bioresorbable. Where the tissue locus to which the morphogen and matrix are applied lacks sufficient endogenous signals to direct the tissue specificity of morphogenesis, the matrix preferably further comprises tissue-specific components or is derived from tissue of the desired type. Matrices can be generated from dehydrated organ-specific tissue by, e.g., treating the tissue with solvents to substantially remove the cellular, non-structural components therefrom. Alternatively, the matrix can be prepared from a biocompatible, *in vivo* biodegradable structural molecule, optionally formulated with suitable tissue-specific cell attachment factors. Thus, collagen, laminin, hyaluronic acid and/or the like, can be used, as can synthetic polymers or copolymers of polylactic acid, polybutyric acid, polyglycolic acid and the like. Currently preferred structural molecules include tissue-specific collagens. Currently preferred cell attachment factors include glycosaminoglycans and proteoglycans. Preferably collagens, glycosaminoglycans and/or proteoglycans are used that are of the same types as those that are naturally found in dental tissues. If needed, the matrix can be treated with an agent effective for enhancing porosity thereof, so as to create a scaffold structure suitable

for cell influx and attachment.

[0015] Alternatively, the morphogen can be applied in association with a carrier that maintains the morphogen substantially at the site of application, and/or enhances the controlled delivery of morphogen substantially at the site at which morphogenesis is to be induced. Such carriers also are disclosed in U.S.S.Nos. 07/971,091 (published as WO94/10203), 08/155,343 (published as WO94/06399) and 08/174,605 (published as WO94/06420). Useful carriers include compositions having a high viscosity, such as that provided by glycerol and the like, as well as carrier materials formulated from extracellular matrices and/or which contain laminin, collagen, and/or biocompatible synthetic polymers, such as polybutyric, polylactic, polyglycolic acids and copolymers thereof.

[0016] Accordingly, the present morphogens can be used to stimulate morphogenesis of new or reparative dentine in a mammalian tooth, including the formation of dentine matrix by mature, differentiated or newly formed odontoblasts, i.e., by competent cells of the tooth pulp tissue. That is, the present morphogens can stimulate proliferation, differentiation and/or phenotypic expression of mammalian cells competent to elaborate dentine matrix, including odontoblasts and/or pulp connective tissue cells. This morphogenetic activity is responsible for the formation of reparative dentine in mammalian teeth. Thus, the present morphogens can be used to increase thickness of a mammalian tooth wall; that is, to increase the thickness of mineralized tissue (dentine, enamel and/or cementum) separating viable tooth pulp tissue from the buccal environment. As a result, the present morphogens can be used to reduce the risk of tooth wall fracture, particularly at sites where the tooth wall is thin or weakened due to association with a gingival lesion site or a cavity.

[0017] Thus, the present invention can be used to seal a tooth cavity, up to and including a Stage V cavity, in a mammalian tooth, particularly a primate tooth such as a human tooth. Carious tissue preferably is ablated from the cavity site to expose a fresh surface of residual dentine therein, preferably transverse to luminae of dental canaliculi within the tooth. The residual dentine surface preferably is located up to about 1 mm, more preferably up to about 0.5 mm, still more preferably up to about 0.2 mm from the pulp chamber wall (i.e., from a mature odontoblast layer at the dentine/pulp interface). Application of a morphogen to this surface prior to or concurrently with tooth reconstruction, including filling of the site of the carious lesion with a suitable material, induces formation of reparative dentine matrix within the reconstructed tooth. In this manner, risk of fracture in the residual dentine, and subsequent treatment by root canal therapy or tooth extraction, can be avoided.

[0018] Similarly, the present invention can be used to desensitize mammalian teeth to perception of pressure and/or temperature in an individual afflicted with periodontal disease, e.g., gingivitis. Following debridement of surfaces within a gingival lesion, including removal of bacterial plaque or tartar, a morphogen is applied to an exposed dentinal surface therein, preferably in an amount effective for stimulating formation of reparative dentine apposite said surface. Reparative dentine so formed can be within or external to the pulp chamber of the treated tooth, and serves as an enhanced protective barrier between the pulp tissue and the buccal environment. Further, morphogen applied to a healthy gingival surface adjoining the lesion site promotes gingival regeneration and/or retards gingival recession.

[0019] In the above-mentioned embodiments, morphogens or morphogen stimulating agents are applied, e.g., topically or by local injection, to a tooth surface e.g., a dentinal surface. Preferably, the surface is transverse to luminae of dental canaliculi within naturally formed tooth dentine, such that fluid microcontact can be established between applied morphogen and odontoblasts or pulp tissue present within the tooth. The morphogen can be applied solubilized or otherwise dispersed (e.g., as a colloidal suspension or emulsion) in a physiologically compatible liquid vehicle, e.g., comprising physiological saline solution, or in a vehicle, e.g., comprising ethanol, that evaporates under physiological conditions to leave a morphogen residue adsorbed on the tooth surface. Alternatively, the morphogen can be sorbed on a matrix such as a biocompatible, acellular matrix suitable for sealing or filling defects in mammalian teeth, e.g., as described above. Morphogen-sealed defects can, if desired, be filled or reconstructed to restore original tooth dimensions using conventional dental reconstruction materials.

[0020] In all such embodiments, the morphogen-treated dentinal surface should be rendered essentially free of buccal microorganisms, and aseptic conditions should be maintained in the treated locus during the time period in which morphogenetic activity is induced.

[0021] Morphogens and morphogen-stimulating agents of the present invention also can be provided to periodontium and/or tooth tissues together with other molecules ("cofactors") known to have a beneficial effect in treating damaged dental tissues, particularly cofactors capable of mitigating or alleviating symptoms typically associated with dental tissue damage and/or loss. Examples of such cofactors include antiseptics such as chlorhexidine and tbezonium iodide, antibiotics, including tetracycline, aminoglycosides, macrolides, penicillins and cephalosporins, anaesthetics and analgesics, and other non-steroidal anti-inflammatory agents.

#### Brief Description of the Drawings

[0022] The foregoing and other objects, features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments, when read together with

the accompanying drawings, in which:

[0023] FIGURE 1 is a schematic illustration of a healthy mammalian tooth in a tooth socket.

[0024] FIGURE 2, panels 2-1 through 2-12, depicts alignment of sequences of various naturally occurring morphogens with a preferred reference sequence of human OP-1, residues 38-139 of Seq. ID No. 4. Morphogens shown in FIGURE 4 also are identified in Table I, above and in the Sequence Listing.

[0025] FIGURE 3 is a digitized video image of a typical tissue section through a primate tooth treated with morphogen, and shows morphogen-induced reparative dentine therein. Bar is 0.5 mm, original magnification 2.5x.

[0026] FIGURE 4 is a bar graph illustration of results establishing that morphogen stimulation of new or reparative dentine formation is dose dependent. In this figure, the dose applied of recombinant human OP-1 is shown in  $\mu\text{g}$  on the X-axis, and the surface area in mm of induced dentine is shown on the Y-axis.

[0027] FIGURE 5 is a line graph illustration of results establishing that morphogen stimulates new or reparative dentine formation under thin bridges of residual natural dentine. In this figure, equivalent amounts (e.g., 10  $\mu\text{g}$ ) of recombinant human OP-1 were applied to residual dentine bridges of the thicknesses shown along the X-axis.

[0028] FIGURE 6 is a line graph illustration of results comparing the effects of recombinant human OP-1 to a conventional agent,  $\text{Ca}(\text{OH})_2$ , on stimulation of new or reparative dentine under thin bridges of residual dentine. Here as well, equivalent amounts (e.g., 10  $\mu\text{g}$ ) of recombinant human OP-1 or  $\text{Ca}(\text{OH})_2$  were applied as indicated to residual dentine bridges of the thicknesses shown on the X-axis.

#### Detailed Description of Preferred Embodiments

[0029] It has been discovered that the morphogens described herein can stimulate tissue formation, including morphogenesis or regeneration of lost or damaged mammalian dental tissue, including dentine. The invention can be used to desensitize teeth, retard gingival recession, seal cavities, increase thickness of the tooth wall, and reduce the risk of tooth wall fracture. The invention is practiced using a morphogen or morphogen-stimulating agent, as defined herein, according to the procedures described herein.

[0030] Provided below is a description of tooth anatomy and useful morphogens, including methods for their production and formulation, as well as exemplary, non-limiting examples which (1) demonstrate the suitability of the morphogens described herein in the methods of the invention, and (2) provide assays with which to test candidate morphogens for their efficacy.

#### I. Tooth Anatomy

[0031] A vertical section of a mammalian tooth in the tooth socket is shown schematically in FIGURE 1. The crown 6 of the tooth is composed of enamel 8 and dentine 22. The pulp chamber 12 is seen in the interior of the crown 6 and the center of the root 10; it extends downward into the bony area 14, 16, 18 and opens by a minute orifice, the apical foramen 20, at the extremity of the root 10. The pulp chamber 12 contains dental pulp, a loose connective tissue richly supplied with blood vessels and nerves, entering the chamber through the apical foramen 20. Some of cells of the pulp tissue, i.e., odontoblasts, the precursors of dentine 22, are arranged generally as a layer on the wall of the pulp chamber 12. During development of the tooth, odontoblasts are columnar, but later, after the dentine 22 is fully formed, they become flattened and resemble osteoblasts.

[0032] The solid portion or mineralized wall of the mature tooth includes dentine 22, enamel 8, and a thin layer of cementum 24, which is disposed on the surface of the root 25. Enamel 8 is formed during development of the tooth from ameloblasts, and cementum 24 is formed from cementoblasts. In a fully developed tooth, the principal mass of the tooth comprises dentine 22, which is made up of hydroxyapatite crystals embedded in a strong meshwork of collagen fibers. The dentine includes a number of minute wavy and branching tubes called dental canaliculi, embedded in a dense homogeneous substance, the matrix. The dental canaliculi are parallel with one another and open at their inner ends into the pulp chamber 12. The dentine matrix is translucent and comprises the majority of the inorganic mass of the dentine. It includes a number of fine fibrils, which are continuous with the fibrils of the dental pulp. After the inorganic matter has been removed by steeping a tooth in weak acid, the remaining organic matter may be torn into laminae that run parallel with the pulp chamber 12 across the direction of the tubes.

[0033] The cementum 24 is disposed as a thin mineralized layer covering the tooth root. It extends from where the enamel terminates to the apex of each root, where it is usually very thick. Cementum resembles bone in structure and chemical composition in that it contains, sparingly, the lacunae and canaliculi that characterize true bone; in the thicker portions of the cementum, the lamellae and Haversian canals peculiar to bone also are found. As a result of aging, the cementum increases in thickness and the pulp chamber also becomes partially filled with a hard substance that is intermediate in structure between dentine and bone (referred to herein as "osteodentine"). It appears to be formed by a slow conversion of the dental pulp, which shrinks or even disappears.

[0034] The periodontal ligament, or periodontal membrane 26, is the layer of periodontal tissue which forms a cushion

between the cementum 24 and the bone 14, 16, 18; it holds the tooth in position by suspending it in the socket (alveolus) of the jawbone. The periodontal ligament is a highly organized tissue which is formed from periodontal fibroblasts. It organizes the collagen fibers which pass directly from the bone of the jaw into the cementum.

[0035] Thus, as used herein, "tooth" refers to a natural or synthetic composition essentially defining the shape of a natural mammalian tooth, having a solid tooth body, including a crown and tooth root. "Periodontium" defines the tissues which surround the tooth in the tooth socket and includes both periodontal ligament and cementum. "Gingiva" defines the dense fibrous tissue, covered by oral mucosa, that envelopes the alveolar bone (tooth socket) processes of the upper and lower jaws, as well as the mineralized tooth wall as it emerges from the periodontium. "Viable" tissue means living, substantially healthy tissue essentially free of microorganisms and infection associated therewith. In particular, viable tissue means viable dental tissue such as enamel, dentine, tooth pulp, gingiva, cementum and periodontal ligament. "Enhancing viability" of dental tissue means improving the structural and functional integrity of living tissue, including improving the clinical status of damaged or diseased tissue. "Viable tooth" refers to an implanted natural tooth with a living tooth root. "Inhibit loss" of dental tissue, as used herein, means inhibiting damage to, and/or loss of, dental tissue and includes regenerating lost, damaged or diseased tissue and/or inhibiting additional damage thereto.

[0036] "Residual dentine" means naturally formed, healthy dentine tissue, e.g., adjoining a carious or gingival lesion, particularly a lesion from which infected dentine has been ablated and/or bacterial plaque or tartar has been debrided. Naturally formed dentine tissue comprises tubules, the dental canaliculi, extending generally radially through the dentine from the layer of odontoblasts lining the pulp chamber wall (described above in connection with FIGURE 1). Thus, a dentinal surface "transverse to the lumina of dental canaliculi" is a dentine surface disposed on any plane that intersects rather than parallels the lumina of one or more dental canaliculi. A "dentinal" surface can define a natural boundary of naturally formed dentine, or a fresh surface of dentine exposed by drilling or other dental techniques, or by fracture or chipping of the tooth wall. A treatment or stimulation "apposite" to a dentinal surface means a treatment or stimulation in juxtaposition or close proximity to the dentinal surface (e.g., separated from said surface by up to about a 1mm thickness of intervening tissue such as residual dentine). "Reparative dentine" comprises atubular dentine matrix elaborated by mature or proliferating odontoblasts or other competent cells of the pulp connective tissue, and can be formed within the pulp chamber of a mammalian tooth.

[0037] "Symptom alleviating cofactor" refers to one or more conventional pharmaceuticals which can, if desired, be included in compositions of this invention and which alleviate or mitigate one or more of the symptoms typically associated with loss of or damage to dental tissue. Exemplary cofactors include antibiotics, antiseptics, non-steroidal anti-inflammatory agents, anaesthetics and analgesics.

## II. Useful Morphogens

[0038] Morphogens useful in this invention include eukaryotic proteins originally identified as osteogenic proteins (see U.S. Patent 5,011,691), such as the OP-1, OP-2, OP-3 and CBMP2 proteins (Seq. ID Nos. 4-9, 15-22, 25 and 26), as well as amino acid sequence-related proteins such as DPP (Seq. ID No. 10, from *Drosophila*), Vgl (Seq. ID No. 11, from *Xenopus*), Vgr-1 (Seq. ID No. 12, from mouse), GDF-1 (Seq. ID Nos. 13, 30 and 31, from humans, see Lee (1991), 88 *PNAS* 4250-4254), 60A (Seq. ID Nos. 23 and 24, from *Drosophila*, see Wharton et al. (1991), 88 *PNAS* 9214-9218), dorsalin-1 (from chick, see Basler et al. (1993), 73 *Cell* 687-702 and GenBank accession number L12032) and GDF-5 (from mouse, see Storm et al. (1994), 368 *Nature* 639-643). Additional useful morphogens include biosynthetic morphogen constructs disclosed in U.S. Pat. No. 5,011,691, e.g., COP-1, 3-5, 7 and 16. See also U.S. Pat. No. 4,968,590.

[0039] Naturally occurring proteins identified and/or appreciated herein to be morphogens form a distinct subgroup within the loose evolutionary grouping of sequence-related proteins known as the TGF $\beta$  superfamily or supergene family. The naturally occurring morphogens share substantial amino acid sequence homology in their C-terminal regions (domains). Typically, the above-mentioned naturally occurring morphogens are translated as a precursor, having an N-terminal signal peptide sequence, typically less than about 30 residues, followed by a "pro" domain that is cleaved to yield the mature C-terminal domain. The signal peptide is cleaved rapidly upon translation, at a cleavage site that can be predicted in a given sequence using the method of Von Heijne (1986), 14 *Nucleic Acids Research* 4683-4691. The pro domain typically is about three times larger than the fully processed mature C-terminal domain. Herein, the "pro" form of a morphogen refers to a morphogen comprising a folded pair of polypeptides each comprising the pro and mature domains of a morphogen polypeptide. Typically, the pro form of a morphogen is more soluble than the mature form under physiological conditions. The pro form appears to be the primary form secreted from cultured mammalian cells.

[0040] Table I, below, summarizes various naturally occurring morphogens identified to date, including their nomenclature as used herein, their Seq. ID references, and publication sources for the amino acid sequences for the full length proteins not included in the Seq. Listing. Each of the generic terms set forth in Table I is intended and should be understood to embrace morphogenically active proteins expressed from nucleic acids encoding the identified se-

quence mentioned below and set forth in the sequence listing, or a morphogenically active fragment or precursor thereof, including functional equivalents thereof such as naturally occurring and biosynthetic variants thereof. Naturally occurring variants thereof include allelic variant forms isolated from other individuals of a single biological species, and phylogenetic counterpart (species) variant forms isolated from phylogenetically distinct biological species.

TABLE I

"OP-1"	Refers generically to morphogenically active proteins expressed from nucleic acid encoding the human OP-1 protein disclosed in Seq. ID No. 4 ("hOP-1"), and includes at least mouse OP-1, Seq. ID No. 5 ("mOP-1"). In each of human and mouse OP-1, Seq. ID Nos. 4 and 5, the conserved seven cysteine skeleton is defined by residues 38 to 139. cDNA sequences and amino acid sequences encoded therein and corresponding to the full length proteins are provided in Seq. ID Nos. 15 and 16 (hOP1) and Seq. ID Nos. 17 and 18 (mOP1.) The mature proteins are defined by residues 293-431 (hOP1) and 292-430 (mOP1). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 30-292 (hOP1) and residues 30-291 (mOP1).
"OP-2"	Refers generically to morphogenically active proteins expressed from a nucleic acid encoding the human OP-2 protein disclosed in Seq. ID No. 6 ("hOP-2"), and includes at least mouse OP-2 ("mOP-2", Seq. ID No. 7). In each of human and mouse OP-2, the conserved seven cysteine skeleton is defined by residues 38 to 139 of Seq. ID Nos. 6 and 7. cDNA sequences and amino acid sequences encoded therein and corresponding to the full length proteins are provided in Seq. ID Nos. 19 and 20 (hOP2) and Seq. ID Nos. 21 and 22 (mOP2.) The mature proteins are defined essentially by residues 264-402 (hOP2) and 261-399 (mOP2). The "pro" regions of the proteins, cleaved to yield the mature, morphogenically active proteins are defined essentially by residues 18-263 (hOP2) and residues 18-260 (mOP1).
"OP-3"	Refers generically to morphogenically active proteins expressed from a nucleic acid encoding the mouse OP-3 protein disclosed in Seq. ID No. 26 ("mOP-3"). The conserved seven cysteine domain is defined by residues 298 to 399 of Seq. ID No. 26, which shares greater than 79% amino acid identity with the corresponding mOP-2 and hOP-2 sequences, and greater than 66% identity with the corresponding OP-1 sequences. A cDNA sequence encoding the above-mentioned amino acid sequence is provided in Seq. ID No. 25. OP-3 is unique among the morphogens identified to date in that the residue at position 9 in the conserved seven cysteine domain (e.g., residue 315 of Seq. ID No. 26) is a serine, whereas other morphogens typically have a tryptophan at this location.
"CBMP2"	Refers generically to morphogenically active proteins expressed from a nucleic acid encoding the CBMP2 proteins, including at least human CBMP2A ("CBMP2A(fx)", Seq ID No. 8) and human CBMP2B ("CBMP2B(fx)", Seq. ID No. 9). The amino acid sequence for the full length proteins, referred to in the literature as BMP2A and BMP2B, or BMP2 and BMP4, appear in Wozney, et al. (1988), 242 <i>Science</i> 1528-1534. The pro domain for BMP2 (BMP2A) likely includes residues 25-248; the mature protein, residues 249-396. The pro domain for BMP4 (BMP2B) likely includes residues 25-256; the mature protein, residues 257-408.
"DPP(fx)"	refers to proteins encoded by the Drosophila DPP gene and defining the conserved seven cysteine skeleton (Seq. ID No. 10). The amino acid sequence for the full length protein appears in Padgett, et al (1987), 325 <i>Nature</i> 81-84. The pro domain likely extends from the signal peptide cleavage site to residue 456; the mature protein likely is defined by residues 457-588.
"Vgl(fx)"	refers to proteins encoded by the Xenopus Vg1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 11). The amino acid sequence for the full length protein appears in Weeks (1987), 51 <i>Cell</i> 861-867. The prodomain likely extends from the signal peptide cleavage site to residue 246; the mature protein likely is defined by residues 247-360.



TABLE I (continued)

5	"Vgr-1(fx)"	refers to proteins encoded by the murine Vgr-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 12). The amino acid sequence for the full length protein appears in Lyons, et al, (1989), 86 <u>PNAS</u> 4554-4558. The prodomain likely extends from the signal peptide cleavage site to residue 299; the mature protein likely is defined by residues 300-438.
10	"GDF-1(fx)"	refers to proteins encoded by the human GDF-1 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 13). The cDNA and encoded amino sequence for the full length protein are provided in Seq. ID. Nos. 30 and 31. The prodomain likely extends from the signal peptide cleavage site to residue 214; the mature protein likely is defined by residues 215-372.
15	"60A"	refers generically to morphogenically active proteins expressed from nucleic acid (e.g., the <i>Drosophila</i> 60A gene) encoding 60A protein or morphogenically active fragments thereof (see Seq. ID Nos. 23 and 24 wherein the cDNA and encoded amino acid sequence for the full length protein are provided). "60A(fx)" refers to the protein sequences defining the conserved seven cysteine skeleton (residues 354 to 455 of Seq. ID No. 24.) The prodomain likely extends from the signal peptide cleavage site to residue 324; the mature protein likely is defined by residues 325-455. The 60A protein is considered likely herein to be a phylogenetic counterpart variant of the human and mouse OP-1 genes; Sampath et al. (1993), 90 <u>PNAS</u> 6004-6008.
20	"BMP3(fx)"	refers to proteins encoded by the human BMP3 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 26). The amino acid sequence for the full length protein appears in Wozney et al. (1988), 242 <u>Science</u> 1528-1534. The pro domain likely extends from the signal peptide cleavage site to residue 290; the mature protein likely is defined by residues 291-472.
25	"BMP5(fx)"	refers to proteins encoded by the human BMP5 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 27). The amino acid sequence for the full length protein appears in Celeste, et al. (1991), 87 <u>PNAS</u> 9843-9847. The pro domain likely extends from the signal peptide cleavage site to residue 316; the mature protein likely is defined by residues 317-454.
30	"BMP6(fx)"	refers to proteins encoded by the human BMP6 gene and defining the conserved seven cysteine skeleton (Seq. ID No. 28). The amino acid sequence for the full length protein appears in Celeste, et al. (1990), 87 <u>PNAS</u> 9843-5847. The pro domain likely includes extends from the signal peptide cleavage site to residue 374; the mature sequence likely includes residues 375-513.
35		

[0041] As shown in FIGURE 2, the OP-2 and OP-3 proteins have an additional cysteine residue in the conserved C-terminal region (e.g., see residue 41 of Seq. ID Nos. 6 and 7), in addition to the conserved cysteine skeleton or domain in common with the other known proteins in this family. The GDF-1 protein has a four amino acid insert within the conserved skeleton (residues 44-47 of Seq. ID No. 13) but this insert likely does not interfere with the relationship of the cysteines in the folded structure. Further, the CBMP2 proteins are missing one amino acid residue within the cysteine skeleton. Thus, these morphogen polypeptides illustrate principles of alignment used herein with respect to the preferred reference morphogen sequence of human OP-1, residues 38-139 of Seq. ID No. 4.

[0042] In certain preferred embodiments, morphogens useful herein include those in which the amino acid sequences of morphogen polypeptides comprise a sequence sharing at least 70% amino acid sequence homology or "similarity", and preferably 80% homology or similarity with a reference morphogen selected from the foregoing naturally occurring morphogens. Preferably, the reference morphogen is human OP-1, and the reference sequence thereof is the C-terminal seven cysteine domain present in morphogenically active forms of human OP-1, residues 38-139 of Seq. ID No. 4. Morphogens useful herein accordingly include allelic, phylogenetic counterpart and other variants of the preferred reference sequence, whether naturally-occurring or biosynthetically produced (e.g., including "muteins" or "mutant proteins"), as well as novel members of the morphogenic family of proteins including the morphogens set forth and identified above, e.g., in Table I. Certain particularly preferred morphogen polypeptides share at least 60% amino acid identity with the preferred reference sequence of human OP-1, still more preferably at least 65% amino acid identity therewith.

[0043] In other preferred embodiments, the family of morphogen polypeptides useful in the present invention, and members thereof, are defined by a generic amino acid sequence. For example, Generic Sequence 7 (Seq. ID No. 1) and Generic Sequence 8 (Seq. ID No. 2) disclosed below, accommodate the homologies shared among preferred

morphogen protein family members identified to date, including at least OP-1, OP-2, OP-3, CBMP2A, CBMP2B, BMP3, 60A, DPP, Vg1, BMP5, BMP6, Vgr-1, and GDF-1 (Seq. ID Nos. 4-15, 24, and 26-29). The amino acid sequences for these proteins are described herein (see Sequence Listing) and/or in the art, as summarized above. The generic sequences include both the amino acid identity shared by these sequences in the C-terminal domain, defined by the six and seven cysteine skeletons (Generic Sequences 7 and 8, respectively), as well as alternative residues for the variable positions within the sequence. The generic sequences provide an appropriate cysteine skeleton where inter- or intramolecular disulfide bonds can form, and contain certain critical amino acids likely to influence the tertiary structure of the folded proteins. In addition, the generic sequences allow for an additional cysteine at position 41 (Generic Sequence 7) or position 46 (Generic Sequence 8), thereby encompassing the morphogenically active sequences of OP-2 and OP-3.

### Generic Sequence 7

			Leu	Xaa	Xaa	Xaa	Phe	Xaa	Xaa	
			1				5			
	Xaa	Gly	Trp	Xaa	Xaa	Xaa	Xaa	Xaa	Pro	
			10				15			
	Xaa	Xaa	Xaa	Xaa	Ala	Xaa	Tyr	Cys	Xaa	Gly
			20				25			
	Xaa	Cys	Xaa	Xaa	Pro	Xaa	Xaa	Xaa	Xaa	Xaa
			30				35			
	Xaa	Xaa	Xaa	Asn	His	Ala	Xaa	Xaa	Xaa	Xaa
			40				45			
	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa	Xaa
			50				55			
	Xaa	Xaa	Xaa	Cys	Cys	Xaa	Pro	Xaa	Xaa	Xaa
			60				65			
	Xaa	Xaa	Xaa	Xaa	Xaa	Leu	Xaa	Xaa	Xaa	Xaa
			70				75			
	Xaa	Xaa	Xaa	Val	Xaa	Leu	Xaa	Xaa	Xaa	Xaa
			80				85			
	Xaa	Met	Xaa	Val	Xaa	Xaa	Cys	Xaa	Cys	Xaa
			90				95			

wherein each Xaa independently is selected from a group of one or more specified amino acids defined as follows: "Res." means "residue" and Xaa at res.2 = (Tyr or Lys); Xaa at res.3 = Val or Ile); Xaa at res.4 = (Ser, Asp or Glu); Xaa at res.6 = (Arg, Gln, Ser, Lys or Ala); Xaa at res.7 = (Asp or Glu); Xaa at res.8 = (Leu, Val or Ile); Xaa at res. 11 = (Gln,

Leu, Asp, His, Asn or Ser; Xaa at res.12 = (Asp, Arg, Asn or Glu); Xaa at res. 13 = (Trp or Ser); Xaa at res.14 = (Ile or Val); Xaa at res.15 = (Ile or Val); Xaa at res.16 (Ala or Ser); Xaa at res.18 = (Glu, Gln, Leu, Lys, Pro or Arg); Xaa at res.19 = (Gly or Ser); Xaa at res.20 = (Tyr or Phe); Xaa at res.21 = (Ala, Ser, Asp, Met, His, Gln, Leu or Gly); Xaa at res.23 = (Tyr, Asn or Phe); Xaa at res.26 = (Glu, His, Tyr, Asp, Gln, Ala or Ser); Xaa at res.28 = (Glu, Lys, Asp, Gln or Ala); Xaa at res.30 = (Ala, Ser, Pro, Gln, Ile or Asn); Xaa at res.31 = (Phe, Leu or Tyr); Xaa at res.33 = (Leu, Val or Met); Xaa at res.34 = (Asn, Asp, Ala, Thr or Pro); Xaa at res.35 = (Ser, Asp, Glu, Leu, Ala or Lys); Xaa at res.36 = (Tyr, Cys, His, Ser or Ile); Xaa at res.37 = (Met, Phe, Gly or Leu); Xaa at res.38 = (Asn, Ser or Lys); Xaa at res.39 = (Ala, Ser, Gly or Pro); Xaa at res.40 = (Thr, Leu or Ser); Xaa at res.44 = (Ile, Val or Thr); Xaa at res.45 = (Val, Leu, Met or Ile); Xaa at res.46 = (Gln or Arg); Xaa at res.47 = (Thr, Ala or Ser); Xaa at res.48 = (Leu or Ile); Xaa at res.49 = (Val or Met); Xaa at res.50 = (His, Asn or Arg); Xaa at res.51 = (Phe, Leu, Asn, Ser, Ala or Val); Xaa at res.52 = (Ile, Met, Asn, Ala, Val, Gly or Leu); Xaa at res.53 = (Asn, Lys, Ala, Glu, Gly or Phe); Xaa at res.54 = (Pro, Ser or Val); Xaa at res.55 = (Glu, Asp, Asn, Gly, Val, Pro or Lys); Xaa at res.56 = (Thr, Ala, Val, Lys, Asp, Tyr, Ser, Gly, Ile or His); Xaa at res.57 = (Val, Ala or Ile); Xaa at res.58 = (Pro or Asp); Xaa at res.59 = (Lys, Leu or Glu); Xaa at res.60 = (Pro, Val or Ala); Xaa at res.63 = (Ala or Val); Xaa at res.65 = (Thr, Ala or Glu); Xaa at res.66 = (Gln, Lys, Arg or Glu); Xaa at res. 67 = (Leu, Met or Val); Xaa at res.68 = (Asn, Ser, Asp or Gly); Xaa at res.69 = (Ala, Pro or Ser); Xaa at res.70 = (Ile, Thr, Val or Leu); Xaa at res.71 = (Ser, Ala or Pro); Xaa at res.72 = (Val, Leu, Met or Ile); Xaa at res.74 = (Tyr or Phe); Xaa at res.75 = (Phe, Tyr, Leu or His); Xaa at res.76 = (Asp, Asn or Leu); Xaa at res.77 = (Asp, Glu, Asn, Arg or Ser); Xaa at res.78 = (Ser, Gln, Asn, Tyr or Asp); Xaa at res.79 = (Ser, Asn, Asp, Glu or Lys); Xaa at res.80 = (Asn, Thr or Lys); Xaa at res.82 = (Ile, Val or Asn); Xaa at res.84 = (Lys or Arg); Xaa at res.85 = (Lys, Asn, Gln, His, Arg or Val); Xaa at res.86 = (Tyr, Glu or His); Xaa at res.87 = (Arg, Gln, Glu or Pro); Xaa at res.88 = (Asn, Glu, Trp or Asp); Xaa at res.90 = (Val, Thr, Ala or Ile); Xaa at res.92 = (Arg, Lys, Val, Asp, Gln or Glu); Xaa at res.93 = (Ala, Gly, Glu or Ser); Xaa at res.95 = (Gly or Ala) and Xaa at res.97 = (His or Arg).

**[0044]** Generic Sequence 8 (Seq. ID No. 2) includes all of Generic Sequence 7 and in addition includes the following sequence (Seq. ID No. 14) at its N-terminus:

**Cys      Xaa      Xaa      Xaa      Xaa**

5

Accordingly, beginning with residue 7, each "Xaa" in Generic Seq. 8 is a specified amino acid defined as for Generic Seq. 7, with the distinction that each residue number described for Generic Sequence 7 is shifted by five in Generic Seq. 8. Thus, "Xaa at res.2 = (Tyr or Lys)" in Gen. Seq. 7 refers to Xaa at res. 7 in Generic Seq. 8. In Generic Seq. 8, Xaa at res.2 = (Lys, Arg, Ala or Gln); Xaa at res.3 = (Lys, Arg or Met); Xaa at res.4 = (His, Arg or Gln); and Xaa at res. 5 = (Glu, Ser, His, Gly, Arg, Pro, Thr, or Tyr).

**[0045]** As noted above, certain currently preferred morphogen polypeptide sequences useful in this invention have greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the preferred reference sequence of hOP-1. These particularly preferred sequences include allelic and phylogenetic counterpart  
40 variants of the OP-1 and OP-2 proteins, including the Drosophila 60A protein. Accordingly, in certain particularly preferred embodiments, useful morphogens include active proteins comprising pairs of polypeptide chains within the generic amino acid sequence herein referred to as "OPX" (Seq. ID No. 3), which defines the seven cysteine skeleton and accommodates the homologies between several identified variants of OP1 and OP2. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal  
45 sequence of mouse or human OP1 or OP2 (see Seq. ID Nos. 4-7 and/or Seq. ID Nos. 15-22).

**[0046]** In still other preferred embodiments, useful morphogen polypeptides have amino acid sequences comprising a sequence encoded by nucleic acid that hybridizes, under stringent hybridization conditions, to DNA or RNA encoding reference morphogen sequences, e.g., C-terminal sequences defining the conserved seven cysteine domains of OP1 or OP2, e.g., nucleotides 1036-1341 and nucleotides 1390-1695 of Seq. ID No. 15 and 19, respectively. As used herein, stringent hybridization conditions are defined as hybridization according to known techniques in 40% formamide, 5 X SSPE, 5 X Denhardt's Solution, and 0.1% SDS at 37°C overnight, and washing in 0.1 X SSPE, 0.1% SDS at 50°C.

**[0047]** As noted above, morphogens useful in the present invention generally are dimeric proteins comprising a folded pair of the above polypeptides. Morphogens are inactive when reduced, but are active as oxidized homodimers and when oxidized in combination with other morphogens of this invention to produce heterodimers. Thus, members of a folded pair of morphogen polypeptides in a morphogenically active protein can be selected independently from any of the specific morphogen polypeptides mentioned above.

**[0048]** The morphogens useful in the methods, compositions and devices of this invention include proteins comprising any of the polypeptide chains described above, whether isolated from naturally-occurring sources, or produced by

recombinant DNA or other synthetic techniques, and includes allelic and phylogenetic counterpart variants of these proteins, as well as biosynthetic variants (muteins) thereof, and various truncated and fusion constructs. Deletion or addition mutants also are envisioned to be active, including those which may alter the conserved C-terminal six or seven cysteine domain, provided that the alteration does not functionally disrupt the relationship of these cysteines in the folded structure. Accordingly, such active forms are considered the equivalent of the specifically described constructs disclosed herein. The proteins may include forms having varying glycosylation patterns, varying N-termini; a family of related proteins having regions of amino acid sequence homology, and active truncated or mutated forms of native or biosynthetic proteins, produced by expression of recombinant DNA in host cells.

**[0049]** The morphogenic proteins can be expressed from intact or truncated cDNA or from synthetic DNAs in prokaryotic or eucaryotic host cells, and purified, cleaved, refolded, and dimerized to form morphogenically active compositions. Currently preferred host cells include *E. coli* or mammalian cells, such as CHO, COS or BSC cells. A detailed description of the morphogens useful in the methods, compositions and devices of this invention is disclosed in co-pending U.S. Serial Nos. 07/752,764 (published as WO92/15323), filed August 30, 1991, and 07/667,724 (abandoned in favor of 07/752,764), filed March 11, 1991.

**[0050]** Thus, in view of this disclosure, skilled genetic engineers can isolate genes from cDNA or genomic libraries of various different biological species, which encode appropriate amino acid sequences, or construct DNAs from oligonucleotides, and then can express them in various types of host cells, including both prokaryotes and eucaryotes, to produce large quantities of active proteins capable of stimulating the morphogenesis of, and/or inhibiting damage to, mammalian dental tissues.

**[0051]** As noted above, a protein is morphogenic herein generally if it induces the developmental cascade of cellular and molecular events that culminate in the formation of new, organ-specific tissue. Preferably, a morphogen comprises a pair of polypeptides having a sequence that corresponds to or is functionally equivalent to at least the conserved C-terminal six or seven cysteine skeleton of human OP-1, included in Seq. ID No. 4. The morphogens generally are competent to induce all of the following biological functions in a morphogenically permissive environment: stimulating proliferation of progenitor cells; stimulating the differentiation of progenitor cells; stimulating the proliferation of differentiated cells; and supporting the growth and maintenance of differentiated cells. Details of how the morphogens useful in this invention first were identified, as well as a description on how to make, use and test them for morphogenic activity are disclosed in U.S.S.Nos. 07/752,764 (published as WO92/15323) and 07/667,724 (abandoned in favor of 07/752,764). As disclosed therein, the morphogens can be purified from naturally-sourced material or recombinantly produced from prokaryotic or eucaryotic host cells, using the genetic sequences disclosed therein. Alternatively, novel morphogenic sequences can be identified following the procedures disclosed therein.

**[0052]** Exemplary useful morphogens include naturally derived proteins comprising a pair of polypeptides, the amino acid sequences of which comprise one or more of the sequences disclosed in the Sequence Listing and FIGURE 2. Other useful sequences include those of the naturally derived morphogens dorsalin-1 and GDF-5, discussed herein in connection with Table I, as well as biosynthetic constructs disclosed in U.S. Pat. 5,011,691 (e.g., COP-1, COP-3, COP-4, COP-5, COP-7, and COP-16).

**[0053]** Accordingly, certain preferred morphogens useful in the methods and compositions of this invention can be described as morphogenically active proteins having amino acid sequences sharing 70% or, preferably, 80% homology (similarity) with a reference morphogen sequence described above, e.g., residues 38-139 of Seq. ID No. 4, where "homology" is as defined herein above. Alternatively, in other preferred embodiments, morphogens useful in the methods and compositions disclosed herein fall within the family of polypeptides described by Generic Sequence 7, Seq. ID No. 1, more preferably by Generic Sequence 8, Seq. ID No. 2.

**[0054]** FIGURE 2 herein sets forth an alignment of the amino acid sequences of the active regions of naturally occurring proteins that have been identified or appreciated herein as morphogens, including human OP-1 (hOP-1, Seq. ID Nos. 4 and 15-16), mouse OP-1 (mOP-1, Seq. ID Nos. 5 and 17-18), human and mouse OP-2 (Seq. ID Nos. 6, 7, and 19-22), mouse OP-3 (Seq. ID Nos. 25-26), CBMP2A (Seq. ID No. 8), CBMP2B (Seq. ID No. 9), BMP3 (Seq. ID No. 27), DPP (from *Drosophila*, Seq. ID No. 10), Vg1, (from *Xenopus*, Seq. ID No. 11), Vgr-1 (from mouse, Seq. ID No. 12), GDF-1 (from mouse and/or human, Seq. ID Nos. 13, 30 and 31), 60A protein (from *Drosophila*, Seq. ID Nos. 23 and 24), BMP5 (Seq. ID No. 28) and BMP6 (Seq. ID No. 29). The sequences are aligned essentially following the method of Needleman et al. (1970), 48 J. Mol. Biol., 443-453, calculated using the Align Program (DNASTar, Inc). In FIGURE 2, three dots indicates that the amino acid in that position is the same as the corresponding amino acid in hOP-1. Three dashes indicates that no amino acid is present in that position, and are included for purposes of illustrating homologies. For example, amino acid residue 60 of CBMP-2A and CBMP-2B is "missing". Of course, both of these amino acid sequences in this region comprise Asn-Ser (residues 58, 59), with CBMP-2A then comprising Lys and Ile, whereas CBMP-2B comprises Ser and Ile. FIGURE 2 also illustrates the handling of insertions in the morphogen amino acid sequence: between residues 56 and 57 of BMP3 is an inserted Val residue; between residues 43 and 44 of GDF-1 is inserted the amino acid sequence, Gly-Gly-Pro-Pro. Such deviations from the reference morphogen sequence are ignored for purposes of calculating the defined relationship between, e.g., GDF-1 and hOP-1. As is apparent from the

amino acid sequence comparisons set forth in FIGURE 4, significant amino acid changes can be made from the reference sequence while retaining morphogenic activity. For example, while the GDF-1 protein sequence depicted in FIGURE 4 shares only about 50% amino acid identity with the hOP1 sequence described therein, the GDF-1 sequence shares greater than 70% amino acid sequence homology (or "similarity") with the hOP1 sequence, where "homology" or "similarity" includes allowed conservative amino acid substitutions within the aligned sequence, e.g., as defined by Dayhoff et al., (1979) 5 Atlas of Protein Sequence and Structure Suppl. 3, pp.345-362, (M.O. Dayhoff, ed., Nat'l BioMed. Res. Fd'n, Washington D.C.).

[0055] The currently most preferred protein sequences useful as morphogens in this invention include those having greater than 60% identity, preferably greater than 65% identity, with the amino acid sequence defining the conserved six or seven cysteine skeleton of hOP1 (e.g., residues 43-139 or 38-139 of Seq. ID No. 5). These most preferred sequences include both allelic and phylogenetic counterpart variants of the OP-1 and OP-2 proteins, including the *Drosophila* 60A protein. Accordingly, in still another preferred aspect, the invention includes morphogens comprising species of polypeptide chains having the generic amino acid sequence referred to herein as "OPX", which defines the seven cysteine domain and accommodates the identities and homologies between the various identified OP1 and OP2 proteins. OPX is presented in Seq. ID No. 3. As described therein, each Xaa at a given position independently is selected from the residues occurring at the corresponding position in the C-terminal sequence of mouse or human OP1 or OP2 (see FIGURE 2 and Seq. ID Nos. 4-7 and/or Seq. ID Nos. 15-22).

[0056] Alternatively, an effective amount of an agent competent to stimulate endogenous morphogen levels in a mammal may be administered by any of the routes described herein. For example, an agent competent to stimulate morphogen production and/or secretion from periodontal tissue, gingiva, alveolar bone tissue in the tooth socket, or pulp tissue, may be provided to a mammal, e.g., by direct administration of the morphogen-stimulating agent to dental tissue. Alternatively, the morphogen-stimulating agent may induce morphogen expression and/or secretion at a distant site (e.g., at a tissue locus other than dental tissue), with the expressed morphogen circulating to dental tissue competent to take up the morphogen and respond thereto. A method for identifying and testing agents competent to modulate the levels of endogenous morphogens in a given tissue is described in detail in prior related U.S.S.Nos. 07/938,021 (published as WO93/05172) and 07/752,859 (published as WO93/05751). Briefly, candidate compounds can be identified and tested by incubation *in vitro* with a test tissue or cells thereof, or a cultured cell line derived therefrom, for a time sufficient to allow the compound to affect the production, i.e., the expression and/or secretion, of a morphogen produced by the cells of that tissue. Here, suitable tissue, or cultured cells of a suitable tissue, preferably can be selected from renal epithelium, dental fibroblasts, cementoblasts, odontoblasts and osteoblasts.

### III. Formulations and Methods for Administration

[0057] The morphogens can be provided to a dental tissue surface, e.g., a dentinal or gingival surface, by any suitable means. Preferably, the morphogen, or a morphogen-stimulating agent, is provided directly to the tissue surface by topical administration. Alternatively, the morphogen can be provided to the tissue by, for example, local injection. While not currently preferred, systemic injection also may be a viable administration route under certain circumstances, such as to treat advanced or chronic disease states, or as a preventive measure in individuals at extreme risk of disease. A detailed description of considerations for systemic administration, including oral and parenteral administration, is disclosed, for example in U.S.S.No. 08/165,511 (published as WO93/04692).

[0058] Where the morphogen is provided directly to a dentinal surface, it can be administered as part of a biocompatible formulation that may be a liquid, gel or solid. For example, it can be dispersed in an aqueous medium that does not impair the mammal's physiologic fluid or salt balance. The aqueous medium for the morphogen thus may comprise normal physiologic saline (0.85% or 0.15 M NaCl), pH 7.0-7.4. The aqueous morphogen formulation can be made, for example, by dissolving the morphogen in 50% ethanol containing acetonitrile in 0.1% trifluoroacetic acid (TFA) or 0.1% HCl, or equivalent solvents. One volume of the resultant solution then is added, for example, to ten volumes of phosphate buffered saline (PBS), which further can include 0.1-0.2% human serum albumin (HSA) or another acceptable carrier protein. The resultant solution preferably is vortexed extensively. The morphogen further can be dispersed in and associated with a carrier capable of maintaining the morphogen at the administered locus. Useful formulations for some embodiments herein include viscous compositions and evaporative compositions. Biocompatible compositions that increase viscosity of the formulation include glycerol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes, and the like. Useful evaporative compositions include physiologically acceptable, e.g., biologically inert, liquids that evaporate under physiological conditions so as to leave a residue of morphogen on the tissue surface. Evaporative liquids include low molecular weight organic or inorganic compounds such as water, ethanol, isopropanol, acetic acid and the like that do not adversely affect tissue function or tissue structural integrity prior to evaporating.

[0059] The formulation also can include an *in vivo* bioresorbable carrier material that acts as a controlled release delivery vehicle. Useful carriers can include biocompatible, preferably biodegradable structural components from, e.

g., an extracellular matrix, such as collagen, laminin, hyaluronic acid, and the like, or polymeric materials, such as polylactic, polybutyric and polyglycolic acids. The carrier also can comprise an acellular tissue matrix, substantially depleted in nonstructural components, such as a demineralized, guanidine-extracted bone, dentine, periodontal ligament or cementum matrix. Details for preparing such matrices are disclosed in U.S.S.N. 07/752,764 (published as WO92/15323). Other useful controlled release carriers in which the morphogen can be dispersed are described in U.S. Pat. Nos. 4,975,526 and 4,919,939. Such carriers are envisioned to be particularly useful where the morphogen is used to seal a cavity.

[0060] Preferably, morphogen compositions that are viscous, evaporative or comprise a bioresorbable carrier are suitable for topical administration to a dentinal or gingival surface, and can inhibit recession or enhance regenerative healing of gingival tissue as well as stimulating morphogenesis of dentine tissue.

[0061] If desired, a given morphogen can be made more soluble in the aqueous composition by association with a suitable molecule. For example, the pro form of a morphogenic protein typically is more soluble or dispersible in physiological solutions than the corresponding mature form. In fact, endogenous morphogens are thought to be transported (e.g., secreted and circulated) in the mammalian body in this form. This soluble form of the protein can be obtained from culture medium of morphogen-secreting mammalian cells e.g., cells transfected with nucleic acid encoding and competent to express the morphogen. Alternatively, a soluble species can be formulated by complexing the mature dimer (or an active fragment thereof) with a morphogen pro domain or a solubility-enhancing fragment thereof (described more fully below). Other components, including various serum proteins, also can be useful to enhance morphogen solubility.

[0062] Finally, the morphogens or morphogen-stimulating agents provided herein can be administered alone or in combination with other molecules, particularly symptom alleviating cofactors. Useful pharmaceutical cofactors for mitigating symptoms associated with damage to dental tissue include antiseptics, antibiotics, anaesthetics and analgesics. Preferred antiseptics for use in the present system include chlorhexidine and tbezonium iodide; preferred antibiotics include tetracycline, aminoglycosides such as neomycin, gentamycin, kanamycin, tobramycin, netilmicin, sisomicin, amikacin, their sulfates or other derivatives, macrolides such as erythromycin, its salts and other derivatives, spiramycin, josamycin or mioramicin, penicillins such as ampicillin, amoxicillin and the like, and cephalosporins, for example, cefaclor, cefadroxil, cefazolin, cefoperazone, cefotaxime, cephalothin, cefalexin, ceforanide, cefonicide or ceftriaxone. Preferred anaesthetics/analgesics include amide-type local anaesthetics such as lidocaine, mepivacaine, pyrocaine, bupivacaine, prilocaine, etidocaine, or other widely used anaesthetics such as procaine.

[0063] Other cofactors include non-steroidal anti-inflammatory agents. However, the morphogens described herein themselves can modulate the body's inflammatory/immune response to an initial tissue injury. Specifically, and as described in detail in U.S.S.N. 08/165,511 (published as WO93/04692), in the presence of a morphogen, proinflammatory effector cells induced to migrate to a site of tissue injury do not become significantly activated. Without being limited to any given theory, it is thought that, in the presence of the morphogen, damaged tissue is induced to undergo a recapitulation of tissue morphogenesis, where progenitor cells are induced to proliferate and differentiate in a tissue-specific manner, and new, functional, organized tissue is formed to replace the damaged or lost tissue, rather than disorganized, fibrous scar tissue.

[0064] The formulated compositions contain therapeutically effective amounts of the morphogen, e.g., amounts which provide appropriate concentrations of the morphogen to the dentinal surface for a time sufficient to stimulate morphogenesis of dentine and/or production of dentine matrix apposite thereto. As will be appreciated by those skilled in the art, the concentration of the compounds described in a therapeutic composition of the present invention will vary depending upon a number of factors, including the biological efficacy of the selected morphogen, the chemical characteristics (e.g., hydrophobicity) of the compounds employed, the formulation of the compound excipients, the administration route, and the treatment envisioned, including whether reparative dentine is to be induced at a distance, e.g., up to about 0.5mm, from the site of application. The preferred dosage to be administered also is likely to depend on such variables such as the condition of the dental tissues particularly of the dentinal surface to which morphogen is to be applied, the size of the tooth or dentinal surface to be treated, extent of dental tissue loss or recession, and the overall health status of the particular patient. In general, 0.00001-1000 mg of morphogen are sufficient with 0.0001-100 mg being preferable and 0.001 to 10 mg being even more preferable for primate teeth, including human teeth. No obvious morphogen induced pathological lesions arise when mature morphogen (e.g., OP-1, 20 mg) is administered daily to normal growing rats for 21 consecutive days. Moreover, 10 mg systemic injections of morphogen (e.g., OP-1) injected daily for 10 days into normal newborn mice does not produce any gross abnormalities.

[0065] Practice of the invention, including additional preferred aspects and embodiments thereof, will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the invention in any way.

## IV. Examples

## Example 1: Morphogen-Induced Dentinogenesis in Mammalian Teeth

**[0066]** The following studies demonstrate the efficacy of morphogens in inducing dentine tissue morphogenesis in model mammals. Human dental pulp has been observed to respond unpredictably to injury. Currently, this represents a basic clinical problem in dentistry. Accordingly, primates are used herein as model mammals for demonstration of dentine regeneration. Those skilled in the dental arts will understand and appreciate the correlation between human and nonhuman primate dental biology.

**[0067]** Recombinant human osteogenic protein-1 (hOP-1, Seq. ID No. 4), when applied to freshly cut primate residual dentine, stimulated significantly more reparative dentine formation than calcium hydroxide paste (a conventional treatment). The response to OP-1 was dependent upon the concentration applied to the tooth as a cavity liner as well as the thickness of the residual dentine. The response to calcium hydroxide similarly was dependent upon the thickness of residual dentine.

**[0068]** Dentine matrices have been shown to contain bone morphogenetic protein (BMP) activity (Bang and Urist (1967), **94 Arch. Surg.** 781-789; Youmans and Urist (1967), **12 Arch. Oral. Biol.** 999-1008; Butler *et al.* (1977), **56 J. Dent. Res.** 288-232; Bessho *et al.* (1990), **70 J. Dent. Res.** 171-175), growth factors (Finkleman *et al.* (1990), **5 J. Bone Min. Res.** 717-723) and dentinogenic activity (Anneroth and Bang (1972), **23 Odont. Rev.** 315-328; Nakashima, M. (1989), **5 Endodont. Dent. Traumat.** 279-286; Nakashima, M. (1990), **35 Arch. Oral. Biol.** 493-497; Nakashima, M. (1990), **35 Arch. Oral. Biol.** 277-281; Tziafas and Kolokuris (1990), **69 J. Dent. Res.** 75-81; Tziafas *et al.* (1992), **37 Arch. Oral. Biol.** 119-128; Smith *et al.* (1994), **39 Arch. Oral. Biol.** 13-22). Impure extracts of dentine with BMP activity (Nakashima, M. (1990), **35 Arch. Oral. Biol.** 493-497; Nakashima, M. (1990), **35 Arch. Oral. Biol.** 277-281), recombinant BMP-2, and BMP-4 (Nakashima, M. (1994), **73 J. Dent. Res.** 1515-1522) and recombinant human osteogenic protein-1 (OP-1, BMP-7) (Rutherford *et al.* (1993), **38 Arch. Oral. Biol.** 571-576; Rutherford *et al.* (1994), **39 Arch. Oral. Biol.** 833-838) induce reparative dentineogenesis when placed on partially amputated pulps in mature adult teeth, see also U.S.S.Nos. 07/752,764 (published as WO92/15323) and 08/155,343 (published as WO94/06399). In addition, dental pulps (Vaino *et al.* (1993), **75 Cell.** 45-58; Heikinheimo, H. (1994), **73 J. Dent. Res.** 590-597) or cells derived from dental pulps (Takeda *et al.* (1994), **15 Bone** 467-470) differentially express some morphogen genes. Accordingly, the present study explored whether solubilized OP-1 induced dentine formation when placed on freshly cut dentine surfaces in monkey permanent teeth.

**[0069]** Ninety (90) incisor, premolar and molar permanent teeth were anesthetized with Carbocaine (Cook-Waite) without vasoconstrictor, isolated by rubber dam, cleaned with a coolant. The variation in the area of the pulpal floors was less than 10% and the mean thickness of the residual floor dentine varied from approximately 0.1 to 0.9 mm between different teeth (as measured histomorphometrically). The pulpal floors were covered a fixed volume of an evaporative solution containing 0.01, 0.1, 1 or 10µg OP-1 in acid-alcohol (28.5% ethanol, 0.025% HCL), acid-alcohol alone, a thin layer of calcium hydroxide paste (Dycal, L.D. Caulk, Wilmington DE) or filled without a liner (no treatment). The cavities were filled with Ketac Silver (ESPE-Premier, Norristown, PA) according to standard reconstructive techniques. It will be recognized that any standard dental reconstructive material could be used. The animals were euthanized two months following surgery, specimens obtained and analyzed as described in the literature (Rutherford *et al.* (1993), **38 Arch. Oral. Biol.** 571-576).

**[0070]** All procedures described above and involving animals were approved by and performed in an accredited animal care facility with extensive experience managing non-human primates. These studies were conducting using 5 adolescent (mixed dentition) male *Macaca fascicularis* of approximately 4-6 kg each. Dental procedures were performed on animals heavily sedated with, e.g., ketamine (15 mg/kg body wt.) and acepromazine (0.55 mg/kg body wt) supplemented with local intraoral infiltration anesthesia (without vasoconstrictor).

**[0071]** The variable amounts of reparative dentine observed in this study typically were limited in area to the dentinal surface transverse to the luminae of cut dentinal canaliculi. FIGURE 3 is a digitized video image of a typical tissue section prepared from an OP-1 treated animal by standard histological techniques. FIGURE 3 shows that reparative dentine formed deep to those dentinal canaliculi cut during preparation of the tooth. In most cases, the reparative dentine was present in all sections in which both the pulpal floor of the cavity preparation and the subjacent pulp chamber were evident. The spatial relationship of the mass of reparative dentine to the pulpal floor appeared to be governed by the orientation of the dentinal canaliculi to the long axis of the tooth and to the surface area of cut dentine intersecting the canaliculi. This spatial orientation suggests that OP-1 diffused through the dentinal canaliculi.

**[0072]** Indeed, the area of new dentine formation two months after morphogen treatment further depended on the dose of OP-1 applied. FIGURE 4 shows histomorphometric results illustrating this relationship. The mean thickness of the residual dentine was determined by averaging three separate and representative histomorphometric measurements in each of 5 sections distributed over 75% of the surface area of the cavity preparation. In FIGURE 4, the mean area of reparative dentine was determined by averaging three replicate histomorphometric measurements in each of

five (5) tissue sections distributed over 75% of the surface area of the cavity preparation. In contrast, there were no significant differences between the amount of reparative dentine deep to the cut dentinal canaliculi in teeth to which no liners were applied (no treatment) and those treated with evaporative carrier alone.

[0073] As shown in FIGURES 5 and 6, equivalent amounts of OP-1 (e.g., 10 $\mu$ g in fixed equivalent volumes per tooth) stimulated significantly more reparative dentine two months after treatment than all other treatments attempted, including calcium hydroxide. The degree of stimulation related to the thickness of residual dentine separating the site of morphogen application from the pulp chamber wall, and became particularly evident as the thickness of residual dentine approached 0.2 mm. Each graphed residual dentine value (0.2, 0.45, 0.75 and 0.9 mm) represents a group of calculated values which ranged up to  $\pm$  0.15mm. Thus, the area of reparative dentine present two (2) months after lining the cavities with 10  $\mu$ g OP-1, a thin layer of calcium hydroxide, or evaporative carrier alone is expressible as a function of the thickness of the residual dentine remaining in the pulpal floor. More reparative dentine was present in OP-1 treated than calcium hydroxide treated teeth (ANOVA, Scheffe's F,  $P < 0.05$ ), in calcium hydroxide than carrier treated teeth ( $P < 0.05$ ), and in OP-1 than carrier treated teeth ( $P < 0.01$ ). OP-1 at 1 $\mu$ g and calcium hydroxide were equipotent over the range of thicknesses of residual dentine (not shown). Smaller amounts of OP-1 were poorly effective in cavities of the size assessed in this study.

[0074] Resection of the dentinal canaliculi may result in odontoblast death, particularly in the deeper preparations (Lee *et al.* (1992), *AM. J. Den.* 64-68). However, it is possible that the tooth preparation procedure utilized preserved odontoblasts even in the deepest preparations (Smith *et al.* (1994), *39 Arch. Oral. Biol.* 13-22). Hence, the dentine formed in these studies may be reactionary dentine, formed by stimulation of the phenotypic function the original odontoblasts, or reparative dentine formed by newly differentiated cells deep to the lost odontoblasts (Lesot *et al.* (1993), *3 Cells and Materials* 201-217; Smith *et al.* (1994), *39 Arch. Oral. Biol.* 13-22). The design utilized in these studies did not permit temporal observations of the odontoblast layer deep to the cut dentinal canaliculi. Earlier studies demonstrated the capacity of OP-1 complexed to an insoluble collagen-based carrier to stimulate reparative dentine when placed directly upon partially amputated pulps (U.S.S.N. 08/155,343 (published as WO94/06399) and Rutherford *et al.* (1993), *38 Arch. Oral. Biol.* 571-576; Rutherford *et al.* (1994), *39 Arch. Oral. Biol.* 833-838). Partial pulp amputation obviously removes the layer of odontoblasts, exposing the deeper fibrous connective tissue of the pulp. Human pulp cells are responsive to OP-1 *in vitro*, further suggesting that pulp itself contains responsive (competent) cells. The specific phenotypes of these OP-1 responsive pulp cells have not yet been identified conclusively.

## Example 2. Preparation of Soluble Morphogen Complexes useful in Stimulating Dentineogenesis

[0075] A currently preferred form of the morphogen useful herein, having improved solubility in aqueous solutions, is a dimeric morphogenic protein comprising at least the C-terminal seven cysteine domain characteristic of the morphogen family, complexed with a peptide comprising a pro region of a member of the morphogen family, or a solubility-enhancing fragment thereof, or an allelic, species or other sequence variant thereof. Preferably, the dimeric morphogenic protein is complexed with two pro region peptides. Also, the dimeric morphogenic protein preferably is noncovalently complexed with the pro region peptides. The pro region peptides preferably comprise at least the N-terminal eighteen amino acids that define the pro domain of a given naturally occurring morphogen, or an allelic or phylogenetic counterpart variant thereof. In other preferred embodiments, peptides defining substantially the full length pro domain are used.

[0076] Other soluble forms of morphogens include dimers of the uncleaved pro forms of these proteins, as well as "hemi-dimers" wherein one subunit of the dimer is an uncleaved pro form of the protein, and the other subunit comprises the mature form of the protein, including truncated forms thereof, preferably noncovalently associated with a cleaved pro domain peptide.

[0077] As described above and in U.S.S.N. 08/040,510 (published as WO94/03600, useful pro domains include the full length pro regions, as well as various truncated forms hereof, particularly truncated forms cleaved at proteolytic Arg-Xaa-Xaa-Arg (Seq. ID No. 32) cleavage sites within the pro domain polypeptide. For example, in OP-1, possible pro sequences include sequences defined by residues 30-292 (full length form); 48-292; and 158-292. Soluble OP-1 complex stability is best enhanced when the pro region comprises the full length form rather than a truncated form, such as the residues 48-292 truncated form, in that residues 30-47 show sequence homology to the N-terminal portions of other morphogens, and currently are believed to have particular utility in enhancing complex stability for all morphogens. Accordingly, currently preferred pro domains include peptides comprising at least the N-terminal fragment, e.g., amino acid residues 30-47 of a naturally occurring morphogen pro domain, or a biosynthetic variant thereof that retains the solubility and/or stability enhancing properties of the naturally-occurring peptide.

[0078] As will be appreciated by those having ordinary skill in the art, useful sequences encoding the pro region can be obtained from genetic sequences encoding known morphogens. Alternatively, chimeric pro regions can be constructed from the sequences of one or more known morphogens. Still another option is to create a synthetic sequence variant of one or more known pro region sequences.



[0079] In another preferred aspect, useful pro region peptides include polypeptide chains comprising an amino acid sequence encoded by a nucleic acid that hybridizes under stringent conditions with a DNA or RNA sequence encoding at least the N-terminal eighteen amino acids of the pro region sequence for OP1 or OP2, e.g., nucleotides 136-192 and 152-211 of Seq. ID No. 15 and 19, respectively.

## 2.1 Isolation of soluble morphogen complex from conditioned media or body fluid

[0080] Morphogens are expressed from mammalian cells as soluble complexes. Typically, however the complex is disassociated during purification, generally by exposure to denaturants often added to the purification solutions, such as detergents, alcohols, organic solvents, chaotropic agents and compounds added to reduce the pH of the solution. Provided below is a currently preferred protocol for purifying the soluble proteins from conditioned media (or, optionally, a body fluid such as serum, cerebrospinal or peritoneal fluid), under non-denaturing conditions. The method is rapid, reproducible and yields isolated soluble morphogen complexes in substantially pure form.

[0081] Soluble morphogen complexes can be isolated from conditioned media using a simple, three step chromatographic protocol performed in the absence of denaturants. The protocol involves running the media (or body fluid) over an affinity column, followed by ion exchange and gel filtration chromatographies. The affinity column described below is a Zn-IMAC column. The present protocol has general applicability to the purification of a variety of morphogens, all of which are anticipated to be isolatable using only minor modifications of the protocol described below. An alternative protocol also envisioned to have utility includes an immunoaffinity column, created using standard procedures and, for example, using antibody specific for a given morphogen pro domain (complexed, for example, to a protein A-conjugated Sepharose column). Protocols for developing immunoaffinity columns are well described in the art, (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly sections VII and XI thereof).

[0082] In this study, OP-1 was expressed in mammalian (CHO, chinese hamster ovary) cells as described in the art (see, for example, international application US90/05903 (WO91/05802). The CHO cell conditioned media containing 0.5% FBS was initially purified using Immobilized Metal-Ion Affinity Chromatography (IMAC). The soluble OP-1 complex from conditioned media binds very selectively to the Zn-IMAC resin and a high concentration of imidazole (50 mM imidazole, pH 8.0) is required for the effective elution of the bound complex. The Zn-IMAC step separates the soluble OP-1 from the bulk of the contaminating serum proteins that elute in the flowthrough and 35 mM imidazole wash fractions. The Zn-IMAC purified soluble OP-1 is next applied to an S-Sepharose cation-exchange column equilibrated in 20 mM NaPO<sub>4</sub> (pH 7.0) with 50 mM NaCl. This S-Sepharose step serves to further purify and concentrate the soluble OP-1 complex in preparation for the following gel filtration step. The protein was applied to a Sephacryl S-200HR column equilibrated in TBS. Using substantially the same protocol, soluble morphogens also can be isolated from one or more body fluids, including serum, cerebrospinal fluid or peritoneal fluid.

[0083] IMAC was performed using Chelating-Sepharose (Pharmacia) that had been charged with three column volumes of 0.2 M ZnSO<sub>4</sub>. The conditioned media was titrated to pH 7.0 and applied directly to the Zn-IMAC resin equilibrated in 20 mM HEPES (pH 7.0) with 500 mM NaCl. The Zn-IMAC resin was loaded with 80 mL of starting conditioned media per mL of resin. After loading, the column was washed with equilibration buffer and most of the contaminating proteins were eluted with 35 mM imidazole (pH 7.0) in equilibration buffer. The soluble OP-1 complex then is eluted with 50 mM imidazole (pH 8.0) in 20 mM HEPES and 500 mM NaCl.

[0084] The 50 mM imidazole eluate containing the soluble OP-1 complex was diluted with nine volumes of 20 mM NaPO<sub>4</sub> (pH 7.0) and applied to an S-Sepharose (Pharmacia) column equilibrated in 20 mM NaPO<sub>4</sub> (pH 7.0) with 50 mM NaCl. The S-Sepharose resin was loaded with an equivalent of 800 mL of starting conditioned media per mL of resin. After loading the S-Sepharose column was washed with equilibration buffer and eluted with 100 mM NaCl followed by 300 mM and 500 mM NaCl in 20 mM NaPO<sub>4</sub> (pH 7.0). The 300 mM NaCl pool was further purified using gel filtration chromatography. Fifty mls of the 300 mM NaCl eluate was applied to a 5.0 X 90 cm Sephacryl S-200HR (Pharmacia) equilibrated in Tris buffered saline (TBS), 50 mM Tris, 150 mM NaCl (pH 7.4). The column was eluted at a flow rate of 5 mL/minute collecting 10 mL fractions. The apparent molecular of the soluble OP-1 was determined by comparison to protein molecular weight standards (alcohol dehydrogenase (ADH, 150 kDa), bovine serum albumin (BSA, 68 kDa), carbonic anhydrase (CA, 30 kDa) and cytochrome C (cyt C, 12.5 kDa). The purity of the S-200 column fractions was determined by separation on standard 15% polyacrylamide SDS gels stained with coomassie blue. The identity of the mature OP-1 and the pro-domain was determined by N-terminal sequence analysis after separation of the mature OP-1 from the pro-domain using standard reverse phase C18 HPLC.

[0085] The soluble OP-1 complex elutes with an apparent molecular weight of 110 kDa. This agrees well with the predicted composition of the soluble OP-1 complex with one mature OP-1 dimer (35-36 kDa) associated with two pro-domains (39 kDa each). Purity of the final complex can be verified by running the appropriate fraction in a reduced 15% polyacrylamide gel.

[0086] The complex components can be verified by running the complex-containing fraction from the S-200 or S-

200HR columns over a reverse phase C18 HPLC column and eluting in an acetonitrile gradient (in 0.1% TFA), using standard procedures. The complex is dissociated by this step, and the pro domain and mature species elute as separate species. These separate species then can be subjected to N-terminal sequencing using standard procedures (see, for example, Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly pp. 602-613), and the identity of the isolated 36kD, 39kDa proteins confirmed as mature morphogen and isolated, cleaved pro domain, respectively. N-terminal sequencing of the isolated pro domain from mammalian cell produced OP-1 revealed 2 forms of the pro region, the intact form (beginning at residue 30 of Seq. ID No. 16) and a truncated form, (beginning at residue 48 of Seq. ID No. 16.) N-terminal sequencing of the polypeptide subunit of the isolated mature species reveals a range of N-termini for the mature sequence, beginning at residues 293, 300, 313, 315, 316, and 318, of Seq. ID No. 16, all of which are active as demonstrated by the standard bone morphogenesis assay set forth in U.S.S.N. 07/752,764 (published as WO92/15323).

## 2.2 *In Vitro* Soluble Morphogen Complex Formation

[0087] As an alternative to purifying soluble complexes from culture media or a body fluid, soluble complexes can be formulated from purified pro domains and mature dimeric species. Successful complex formation apparently requires association of the components under denaturing conditions sufficient to relax the folded structure of these molecules, without affecting disulfide bonds. Preferably, the denaturing conditions mimic the environment of an intracellular vesicle sufficiently such that the cleaved pro domain has an opportunity to associate with the mature dimeric species under relaxed folding conditions. The concentration of denaturant in the solution then is decreased in a controlled, preferably step-wise manner, so as to allow proper refolding of the dimer and pro regions while maintaining the association of the pro domain with the dimer. Useful denaturants include 4-6M urea or guanidine hydrochloride (GuHCl), in buffered solutions of pH 4-10, preferably pH 6-8. The soluble complex then is formed by controlled dialysis or dilution into a solution having a final denaturant concentration of less than 0.1-2M urea or GuHCl, preferably 1-2 M urea of GuHCl, which then preferably can be diluted into a physiological buffer. Protein purification/renaturing procedures and considerations are well described in the art, and details for developing a suitable renaturing protocol readily can be determined by one having ordinary skill in the art. One useful text on the subject is Guide to Protein Purification, M. Deutscher, ed., Academic Press, San Diego, 1990, particularly section V Complex formation also may be aided by addition of one or more chaperone proteins.

## 2.3 Stability of Soluble Morphogen Complexes

[0088] The stability of the highly purified soluble morphogen complex in a physiological buffer, e.g., tris-buffered saline (TBS) and phosphate-buffered saline (PBS), can be enhanced by any of a number of means. Currently preferred is by means of a pro region that comprises at least the first 18 amino acids of the pro sequence (e.g., residues 30-47 of Seq. ID NO. 16 for OP-1), and preferably is the full length pro region. Residues 30-47 show sequence homology to the N-terminal portion of other morphogens and are believed to have particular utility in enhancing complex stability for all morphogens. Other useful means for enhancing the stability of soluble morphogen complexes include three classes of additives. These additives include basic amino acids (e.g., L-arginine, lysine and betaine); nonionic detergents (e.g., Tween 80 or Nonidet P-120); and carrier proteins (e.g., serum albumin and casein). Useful concentrations of these additives include 1-100 mM, preferably 10-70 mM, including 50 mM, basic amino acid; 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (v/v) nonionic detergent; and 0.01-1.0%, preferably 0.05-0.2%, including 0.1% (w/v) carrier protein.

## SEQUENCE LISTING

### [0089]

#### (1) GENERAL INFORMATION:

##### (i) APPLICANT:

(A) NAME: CREATIVE BIOMOLECULES, INC  
 (B) STREET: 45 SOUTH STREET  
 (C) CITY: HOPKINTON  
 (D) STATE: MA  
 (E) COUNTRY: USA  
 (F) POSTAL CODE (ZIP): 01748

(G) TELEPHONE: 1-508-435-9001

(H) TELEFAX: 1-508-435-0454

(I) TELEX:

5 (ii) TITLE OF INVENTION: MORPHOGEN-INDUCED DENTINE REGENERATION

(iii) NUMBER OF SEQUENCES: 32

10 (iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: CREATIVE BIOMOLECULES, INC

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(C) CITY: HOPKINTON

(D) STATE: MA

15 (E) COUNTRY: USA

(F) ZIP: 01748

(v) COMPUTER READABLE FORM:

20 (A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

25 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

30 (C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: FENTON, GILLIAN M

(B) REGISTRATION NUMBER: 36,508

35 (C) REFERENCE/DOCKET NUMBER: CRP-088PC

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (617) 248-7000

40 (B) TELEFAX: (617) 248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

45 (A) LENGTH: 97 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

50 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

55 (A) NAME/KEY: Protein

(B) LOCATION: 1..97

(D) OTHER INFORMATION: /label= Generic-Seq-7

/note= "wherein each Xaa is independently selected from a group of one or more specified amino acids"

as defined in the specification."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

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      Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly Xaa Cys Xaa Xaa Pro
10      20          25          30
      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala Xaa Xaa Xaa Xaa
      35          40          45
      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Cys Cys Xaa Pro
15      50          55          60
      Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa
      65          70          75          80
20      Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Met Xaa Val Xaa Xaa Cys Xaa Cys
      85          90          95
      Xaa

```

25 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

```

30      (A) LENGTH: 102 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS:
      (D) TOPOLOGY: linear

```

35 (ii) MOLECULE TYPE: protein

35 (ix) FEATURE:

```

40      (A) NAME/KEY: Protein
      (B) LOCATION: 1..102
      (D) OTHER INFORMATION: /label= Generic-Seq-8
      /note= "wherein each Xaa is independently selected from a group of one or more specified amino acids
      as defined in the specification."

```

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

45      Cys Xaa Xaa Xaa Xaa Leu Xaa Xaa Xaa Phe Xaa Xaa Xaa Gly Trp Xaa
      1          5          10          15
50      Xaa Xaa Xaa Xaa Xaa Pro Xaa Xaa Xaa Xaa Ala Xaa Tyr Cys Xaa Gly
      20          25          30
      Xaa Cys Xaa Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Asn His Ala
      35          40          45
55

```

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 50 55 60  
 5 Xaa Cys Cys Xaa Pro Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 65 70 75 80  
 Xaa Xaa Xaa Xaa Xaa Val Xaa Leu Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
 85 90 95  
 10 Xaa Xaa Cys Xaa Cys Xaa  
 100

## (2) INFORMATION FOR SEQ ID NO:3:

15

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 20 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

25

## (ix) FEATURE:

(A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= OPX  
 30 /note= "wherein each Xaa is independently selected from a group of one or more specified amino acids  
 as defined in the specification."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

35

Cys Xaa Xaa His Glu Leu Tyr Val Xaa Phe Xaa Asp Leu Gly Trp Xaa  
 1 5 10 15  
 Asp Trp Xaa Ile Ala Pro Xaa Gly Tyr Xaa Ala Tyr Tyr Cys Glu Gly  
 20 25 30  
 Glu Cys Xaa Phe Pro Leu Xaa Ser Xaa Met Asn Ala Thr Asn His Ala  
 35 40 45  
 Ile Xaa Gln Xaa Leu Val His Xaa Xaa Xaa Pro Xaa Xaa Val Pro Lys  
 45 50 55 60  
 Xaa Cys Cys Ala Pro Thr Xaa Leu Xaa Ala Xaa Ser Val Leu Tyr Xaa  
 65 70 75 80  
 50 Asp Xaa Ser Xaa Asn Val Xaa Leu Xaa Lys Xaa Arg Asn Met Val Val  
 85 90 95  
 Xaa Ala Cys Gly Cys His  
 100

55

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

10

(A) ORGANISM: Homo sapiens  
 (F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

15

(A) NAME/KEY: Protein  
 (B) LOCATION: 1..139  
 (D) OTHER INFORMATION: /label= hOP1-MATURE

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

	Ser	Thr	Gly	Ser	Lys	Gln	Arg	Ser	Gln	Asn	Arg	Ser	Lys	Thr	Pro	Lys	
	1				5					10					15		
25	Asn	Gln	Glu	Ala	Leu	Arg	Met	Ala	Asn	Val	Ala	Glu	Asn	Ser	Ser	Ser	
				20					25					30			
	Asp	Gln	Arg	Gln	Ala	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val	Ser	Phe	Arg	
				35					40					45			
30	Asp	Leu	Gly	Trp	Gln	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala	Ala	
		50					55						60				
	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	
35		65				70					75					80	
	Ala	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Phe	Ile	Asn	Pro	
					85					90					95		
	Glu	Thr	Val	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln	Leu	Asn	Ala	Ile	
40				100					105					110			
	Ser	Val	Leu	Tyr	Phe	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys	Lys	Tyr	
			115					120					125				
45	Arg	Asn	Met	Val	Val	Arg	Ala	Cys	Gly	Cys	His						
			130				135										

(2) INFORMATION FOR SEQ ID NO:5:

50

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE  
(F) TISSUE TYPE: EMBRYO

## (ix) FEATURE:

(A) NAME/KEY: Protein  
(B) LOCATION: 1..139  
(D) OTHER INFORMATION: /label= mOP1-MATURE

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```

15      Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys
        1          5          10          15
      Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser
        20          25          30
20      Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg
        35          40          45
      Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala
        50          55          60
25      Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn
        65          70          75          80
      Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro
        85          90          95
30      Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile
        100          105          110
      Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr
        115          120          125
35      Arg Asn Met Val Val Arg Ala Cys Gly Cys His
        130          135

```

## (2) INFORMATION FOR SEQ ID NO:6:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS:  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS  
(F) TISSUE TYPE: HIPPOCAMPUS

## (ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..139

(D) OTHER INFORMATION: /label= HOP2-MATURE

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

5

Ala Val Arg Pro Leu Arg Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu  
1 5 10 15

10

Pro Gln Ala Asn Arg Leu Pro Gly Ile Phe Asp Asp Val His Gly Ser  
20 25 30

15

His Gly Arg Gln Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Gln  
35 40 45

Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala  
50 55 60

20

Tyr Tyr Cys Glu Gly Glu Cys Ser Phe Pro Leu Asp Ser Cys Met Asn  
65 70 75 80

Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro  
85 90 95

25

Asn Ala Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr  
100 105 110

Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His  
115 120 125

30

Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
130 135

(2) INFORMATION FOR SEQ ID NO:7:

35

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 139 amino acids

(B) TYPE: amino acid

40

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

45

(vi) ORIGINAL SOURCE:

(A) ORGANISM: MURIDAE

(F) TISSUE TYPE: EMBRYO

50

(ix) FEATURE:

(A) NAME/KEY: Protein

(B) LOCATION: 1..139

(D) OTHER INFORMATION: /label= MOP2-MATURE

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:



1 Ala Ala Arg Pro Leu Lys Arg Arg Gln Pro Lys Lys Thr Asn Glu Leu  
 5 Pro His Pro Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser  
 Arg Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg  
 10 Asp Leu Gly Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala  
 Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn  
 15 Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro  
 Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr  
 20 Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His  
 25 Arg Asn Met Val Val Lys Ala Cys Gly Cys His

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: bovinæ

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..101  
 (D) OTHER INFORMATION: /label= CBMP-2A-FX

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

1 Cys Lys Arg His Pro Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
 5 5 10 15  
 5 Asp Trp Ile Val Ala Pro Pro Gly Tyr His Ala Phe Tyr Cys His Gly  
 20 25 30  
 Glu Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
 35 40 45  
 10 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Lys Ile Pro Lys Ala  
 50 55 60  
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
 65 70 75 80  
 15 Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Glu  
 85 90 95  
 Gly Cys Gly Cys Arg  
 100

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS  
 (F) TISSUE TYPE: hippocampus

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..101  
 (D) OTHER INFORMATION: /label= CBMP-2B-FX

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

1 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn  
 5 1 5 10 15  
 5 Asp Trp Ile Val Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly  
 20 25 30  
 Asp Cys Pro Phe Pro Leu Ala Asp His Leu Asn Ser Thr Asn His Ala  
 35 40 45  
 10 Ile Val Gln Thr Leu Val Asn Ser Val Asn Ser Ser Ile Pro Lys Ala  
 50 55 60  
 Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu Tyr Leu Asp  
 65 70 75 80  
 15 Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu Met Val Val Glu  
 85 90 95  
 Gly Cys Gly Cys Arg  
 100  
 20

## (2) INFORMATION FOR SEQ ID NO:10:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear  
 30

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

35 (A) ORGANISM: DROSOPHILA MELANOGASTER

## (ix) FEATURE:

- 40 (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= DPP-FX

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

45 Cys Arg Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asp  
 1 5 10 15  
 Asp Trp Ile Val Ala Pro Leu Gly Tyr Asp Ala Tyr Tyr Cys His Gly  
 20 25 30  
 50  
 55

Lys Cys Pro Phe Pro Leu Ala Asp His Phe Asn Ser Thr Asn His Ala  
 35 40 45  
 5 Val Val Gln Thr Leu Val Asn Asn Asn Asn Pro Gly Lys Val Pro Lys  
 50 55 60  
 Ala Cys Cys Val Pro Thr Gln Leu Asp Ser Val Ala Met Leu Tyr Leu  
 65 70 75 80  
 10 Asn Asp Gln Ser Thr Val Val Leu Lys Asn Tyr Gln Glu Met Thr Val  
 85 90 95  
 Val Gly Cys Gly Cys Arg  
 100  
 15

## (2) INFORMATION FOR SEQ ID NO:11:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: XENOPUS

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= VGL-FX

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Lys Lys Arg His Leu Tyr Val Glu Phe Lys Asp Val Gly Trp Gln  
 1 5 10 15  
 Asn Trp Val Ile Ala Pro Gln Gly Tyr Met Ala Asn Tyr Cys Tyr Gly  
 20 25 30  
 45 Glu Cys Pro Tyr Pro Leu Thr Glu Ile Leu Asn Gly Ser Asn His Ala  
 35 40 45  
 Ile Leu Gln Thr Leu Val His Ser Ile Glu Pro Glu Asp Ile Pro Leu  
 50 55 60  
 Pro Cys Cys Val Pro Thr Lys Met Ser Pro Ile Ser Met Leu Phe Tyr  
 65 70 75 80  
 Asp Asn Asn Asp Asn Val Val Leu Arg His Tyr Glu Asn Met Ala Val  
 85 90 95  
 Asp Glu Cys Gly Cys Arg  
 100

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /label= VGR-1-FX

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

```

Cys Lys Lys His Glu Leu Tyr Val Ser Phe Gln Asp Val Gly Trp Gln
1          5          10          15
Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly
20          25          30
Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala
35          40          45
Ile Val Gln Thr Leu Val His Val Met Asn Pro Glu Tyr Val Pro Lys
50          55          60
Pro Cys Cys Ala Pro Thr Lys Val Asn Ala Ile Ser Val Leu Tyr Phe
65          70          75          80
Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val
85          90          95
Arg Ala Cys Gly Cys His
100

```

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens  
 (F) TISSUE TYPE: brain

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..106  
 (D) OTHER INFORMATION: /note= "GDF-1 (fx)"  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

5  
 10 Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp His  
 1 5 10 15  
 Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln Gly  
 20 25 30  
 15 Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro Ala  
 35 40 45  
 Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro Gly  
 50 55 60  
 20 Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile Ser  
 65 70 75 80  
 Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr Glu  
 85 90 95  
 25 Asp Met Val Val Asp Glu Cys Gly Cys Arg  
 100 105

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Xaa Xaa Xaa Xaa  
 1 5

## (2) INFORMATION FOR SEQ ID NO:15:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1822 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS  
(F) TISSUE TYPE: HIPPOCAMPUS

(ix) FEATURE:

5

(A) NAME/KEY: CDS  
(B) LOCATION: 49..1341  
(C) IDENTIFICATION METHOD: experimental  
(D) OTHER INFORMATION: /function=  
"OSTEOGENIC PROTEIN"  
/product= "OP1"  
/evidence= EXPERIMENTAL  
/standard\_name= "OP1"

10

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

20

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35

40

45

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55

	GGTGC	GGGCC	CGGAG	CCCCGG	AGCCCCGGTA	GCGCGTAGAG	CCGGCGCG	ATG	CAC	GTG		57					
								Met	His	Val							
								1									
5	CGC	TCA	CTG	CGA	GCT	GCG	GCG	CCG	CAC	AGC	TTC	GTG	GCG	CTC	TGG	GCA	105
	Arg	Ser	Leu	Arg	Ala	Ala	Ala	Pro	His	Ser	Phe	Val	Ala	Leu	Trp	Ala	
	5						10					15					
10	CCC	CTG	TTC	CTG	CTG	CGC	TCC	GCC	CTG	GCC	GAC	TTC	AGC	CTG	GAC	AAC	153
	Pro	Leu	Phe	Leu	Leu	Arg	Ser	Ala	Leu	Ala	Asp	Phe	Ser	Leu	Asp	Asn	
	20					25					30					35	
15	GAG	GTG	CAC	TCG	AGC	TTC	ATC	CAC	CGG	CGC	CTC	CGC	AGC	CAG	GAG	CGG	201
	Glu	Val	His	Ser	Ser	Phe	Ile	His	Arg	Arg	Leu	Arg	Ser	Gln	Glu	Arg	
					40					45					50		
20	CGG	GAG	ATG	CAG	CGC	GAG	ATC	CTC	TCC	ATT	TTG	GGC	TTG	CCC	CAC	CGC	249
	Arg	Glu	Met	Gln	Arg	Glu	Ile	Leu	Ser	Ile	Leu	Gly	Leu	Pro	His	Arg	
				55				60						65			
25	CCG	CGC	CCG	CAC	CTC	CAG	GGC	AAG	CAC	AAC	TCG	GCA	CCC	ATG	TTC	ATG	297
	Pro	Arg	Pro	His	Leu	Gln	Gly	Lys	His	Asn	Ser	Ala	Pro	Met	Phe	Met	
			70					75					80				
30	CTG	GAC	CTG	TAC	AAC	GCC	ATG	GCG	GTG	GAG	GAG	GGC	GGC	GGG	CCC	GGC	345
	Leu	Asp	Leu	Tyr	Asn	Ala	Met	Ala	Val	Glu	Glu	Gly	Gly	Gly	Pro	Gly	
	85					90						95					
35	GGC	CAG	GGC	TTC	TCC	TAC	CCC	TAC	AAG	GCC	GTC	TTC	AGT	ACC	CAG	GGC	393
	Gly	Gln	Gly	Phe	Ser	Tyr	Pro	Tyr	Lys	Ala	Val	Phe	Ser	Thr	Gln	Gly	
	100					105					110					115	
40	CCC	CCT	CTG	GCC	AGC	CTG	CAA	GAT	AGC	CAT	TTC	CTC	ACC	GAC	GCC	GAC	441
	Pro	Pro	Leu	Ala	Ser	Leu	Gln	Asp	Ser	His	Phe	Leu	Thr	Asp	Ala	Asp	
					120					125					130		
45	ATG	GTC	ATG	AGC	TTC	GTC	AAC	CTC	GTG	GAA	CAT	GAC	AAG	GAA	TTC	TTC	489
	Met	Val	Met	Ser	Phe	Val	Asn	Leu	Val	Glu	His	Asp	Lys	Glu	Phe	Phe	
				135				140						145			
50	CAC	CCA	CGC	TAC	CAC	CAT	CGA	GAG	TTC	CGG	TTT	GAT	CTT	TCC	AAG	ATC	537
	His	Pro	Arg	Tyr	His	His	Arg	Glu	Phe	Arg	Phe	Asp	Leu	Ser	Lys	Ile	
				150				155						160			
55	CCA	GAA	GGG	GAA	GCT	GTC	ACG	GCA	GCC	GAA	TTC	CGG	ATC	TAC	AAG	GAC	585
	Pro	Glu	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Asp	
		165				170						175					
60	TAC	ATC	CGG	GAA	CGC	TTC	GAC	AAT	GAG	ACG	TTC	CGG	ATC	AGC	GTT	TAT	633
	Tyr	Ile	Arg	Glu	Arg	Phe	Asp	Asn	Glu	Thr	Phe	Arg	Ile	Ser	Val	Tyr	
	180					185					190					195	
65	CAG	GTG	CTC	CAG	GAG	CAC	TTG	GGC	AGG	GAA	TCG	GAT	CTC	TTC	CTG	CTC	681
	Gln	Val	Leu	Gln	Glu	His	Leu	Gly	Arg	Glu	Ser	Asp	Leu	Phe	Leu	Leu	
					200					205					210		



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	GAC AGC CGT ACC CTC TGG GCC TCG GAG GAG GGC TGG CTG GTG TTT GAC	729
	Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp	
	215 220 225	
5	ATC ACA GCC ACC AGC AAC CAC TGG GTG GTC AAT CCG CGG CAC AAC CTG	777
	Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu	
	230 235 240	
10	GGC CTG CAG CTC TCG GTG GAG ACG CTG GAT GGG CAG AGC ATC AAC CCC	825
	Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro	
	245 250 255	
15	AAG TTG GCG GGC CTG ATT GGG CGG CAC GGG CCC CAG AAC AAG CAG CCC	873
	Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro	
	260 265 270 275	
20	TTC ATG GTG GCT TTC TTC AAG GCC ACG GAG GTC CAC TTC CGC AGC ATC	921
	Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe Arg Ser Ile	
	280 285 290	
25	CGG TCC ACG GGG AGC AAA CAG CGG AGC CAG AAC CGC TCC AAG ACG CCC	969
	Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro	
	295 300 305	
30	AAG AAC CAG GAA GCC CTG CGG ATG GCC AAC GTG GCA GAG AAC AGC AGC	1017
	Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu Asn Ser Ser	
	310 315 320	
35	AGC GAC CAG AGG CAG GCC TGT AAG AAG CAC GAG CTG TAT GTC AGC TTC	1065
	Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe	
	325 330 335	
40	CGA GAC CTG GGC TGG CAG GAC TGG ATC ATC GCG CCT GAA GGC TAC GCC	1113
	Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala	
	340 345 350 355	
45	GCC TAC TAC TGT GAG GGG GAG TGT GCC TTC CCT CTG AAC TCC TAC ATG	1161
	Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met	
	360 365 370	
50	AAC GCC ACC AAC CAC GCC ATC GTG CAG ACG CTG GTC CAC TTC ATC AAC	1209
	Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn	
	375 380 385	
55	CCG GAA ACG GTG CCC AAG CCC TGC TGT GCG CCC ACG CAG CTC AAT GCC	1257
	Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala	
	390 395 400	
60	ATC TCC GTC CTC TAC TTC GAT GAC AGC TCC AAC GTC ATC CTG AAG AAA	1305
	Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys	
	405 410 415	
65	TAC AGA AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCCTCC	1351
	Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His	
	420 425 430	
70	GAGAATTCAG ACCCTTTGGG GCCAAGTTTT TCTGGATCCT CCATTGCTCG CCTTGGCCAG	1411
75	GAACCAGCAG ACCAACTGCC TTTTGTGAGA CCTTCCCTC CCTATCCCCA ACTTTAAAGG	1471
80	TGTGAGAGTA TTAGGAAACA TGAGCAGCAT ATGGCTTTTG ATCAGTTTTT CAGTGGCAGC	1531

ATCCAATGAA CAAGATCCTA CAAGCTGTGC AGGCAAAACC TAGCAGGAAA AAAAAACAAC 1591  
 GCATAAGAA AAATGGCCGG GCCAGGTCAT TGGCTGGGAA GTCTCAGCCA TGCACGGACT 1651  
 5 CGTTTCCAGA GGTAATTATG AGCGCCTACC AGCCAGGCCA CCCAGCCGTG GGAGGAAGGG 1711  
 GGCGTGGCAA GGGGTGGGCA CATTGGTGTC TGTGCGAAAG GAAAATTGAC CCGGAAGTTC 1771  
 10 CTGTAATAAA TGTACAATA AAACGAATGA ATGAAAAAAA AAAAAAAAAA A 1822

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 431 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
 1 5 10 15  
 5 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
 20 25 30  
 Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
 35 40 45  
 10 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
 50 55 60  
 Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro  
 65 70 75 80  
 15 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Gly Gly  
 85 90 95  
 Gly Pro Gly Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser  
 100 105 110  
 20 Thr Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr  
 115 120 125  
 Asp Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys  
 130 135 140  
 25 Glu Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu  
 145 150 155 160  
 Ser Lys Ile Pro Glu Gly Glu Ala Val Thr Ala Ala Glu Phe Arg Ile  
 165 170 175  
 30 Tyr Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Arg Ile  
 180 185 190  
 35 Ser Val Tyr Gln Val Leu Gln Glu His Leu Gly Arg Glu Ser Asp Leu  
 195 200 205

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Phe Leu Leu Asp Ser Arg Thr Leu Trp Ala Ser Glu Glu Gly Trp Leu  
 210 215 220  
 5 Val Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg  
 225 230 235 240  
 His Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser  
 245 250 255  
 10 Ile Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn  
 260 265 270  
 Lys Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Phe  
 275 280 285  
 15 Arg Ser Ile Arg Ser Thr Gly Ser Lys Gln Arg Ser Gln Asn Arg Ser  
 290 295 300  
 Lys Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Asn Val Ala Glu  
 305 310 315 320  
 Asn Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr  
 325 330 335  
 25 Val Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu  
 340 345 350  
 Gly Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn  
 355 360 365  
 30 Ser Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His  
 370 375 380  
 Phe Ile Asn Pro Glu Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln  
 385 390 395 400  
 35 Leu Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile  
 405 410 415  
 Leu Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His  
 420 425 430

## (2) INFORMATION FOR SEQ ID NO:17:

## 45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1873 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 50 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

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- (A) ORGANISM: MURIDAE  
 (F) TISSUE TYPE: EMBRYO

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 104...1393

(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN".

/product= "MOP1"

/note= "MOP1 (CDNA)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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	CTGCAGCAAG TGACCTCGGG TCGTGGACCG CTGCCCTGCC CCCTCCGCTG CCACCTGGGG	60
5	CGGCGCGGGC CCGGTGCCCC GGATCGCGCG TAGAGCCGGC GCG ATG CAC GTG CGC Met His Val Arg 1	115
10	TCG CTG CGC GCT GCG GCG CCA CAC AGC TTC GTG GCG CTC TGG GCG CCT Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala Leu Trp Ala Pro 5 10 15 20	163
15	CTG TTC TTG CTG CGC TCC GCC CTG GCC GAT TTC AGC CTG GAC AAC GAG Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser Leu Asp Asn Glu 25 30 35	211
20	GTG CAC TCC AGC TTC ATC CAC CGG CGC CTC CGC AGC CAG GAG CGG CGG Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser Gln Glu Arg Arg 40 45 50	259
25	GAG ATG CAG CGG GAG ATC CTG TCC ATC TTA GGG TTG CCC CAT CGC CCG Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu Pro His Arg Pro 55 60 65	307
30	CGC CCG CAC CTC CAG GGA AAG CAT AAT TCG GCG CCC ATG TTC ATG TTG Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro Met Phe Met Leu 70 75 80	355
35	GAC CTG TAC AAC GCC ATG GCG GTG GAG GAG AGC GGG CCG GAC GGA CAG Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly Pro Asp Gly Gln 85 90 95 100	403
40	GGC TTC TCC TAC CCC TAC AAG GCC GTC TTC AGT ACC CAG GGC CCC CCT Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr Gln Gly Pro Pro 105 110 115	451
45	TTA GCC AGC CTG CAG GAC AGC CAT TTC CTC ACT GAC GCC GAC ATG GTC Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp Ala Asp Met Val 120 125 130	499
50	ATG AGC TTC GTC AAC CTA GTG GAA CAT GAC AAA GAA TTC TTC CAC CCT Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu Phe Phe His Pro 135 140 145	547
55	CGA TAC CAC CAT CGG GAG TTC CGG TTT GAT CTT TCC AAG ATC CCC GAG Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser Lys Ile Pro Glu 150 155 160	595
60	GGC GAA CGG GTG ACC GCA GCC GAA TTC AGG ATC TAT AAG GAC TAC ATC Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr Lys Asp Tyr Ile 165 170 175 180	643
65	CGG GAG CGA TTT GAC AAC GAG ACC TTC CAG ATC ACA GTC TAT CAG GTG Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr Val Tyr Gln Val 185 190 195	691
70	CTC CAG GAG CAC TCA GGC AGG GAG TCG GAC CTC TTC TTG CTG GAC AGC Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe Leu Leu Asp Ser 200 205 210	739

	CGC ACC ATC TGG GCT TCT GAG GAG GGC TGG TTG GTG TTT GAT ATC ACA	787
	Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val Phe Asp Ile Thr	
	215 220 225	
5	GCC ACC AGC AAC CAC TGG GTG GTC AAC CCT CGG CAC AAC CTG GGC TTA	835
	Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His Asn Leu Gly Leu	
	230 235 240	
10	CAG CTC TCT GTG GAG ACC CTG GAT GGG CAG AGC ATC AAC CCC AAG TTG	883
	Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile Asn Pro Lys Leu	
	245 250 255 260	
15	GCA GGC CTG ATT GGA CGG CAT GGA CCC CAG AAC AAG CAA CCC TTC ATG	931
	Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys Gln Pro Phe Met	
	265 270 275	
	GTG GCC TTC TTC AAG GCC ACG GAA GTC CAT CTC CGT AGT ATC CGG TCC	979
	Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg Ser Ile Arg Ser	
	280 285 290	
20	ACG GGG GGC AAG CAG CGC AGC CAG AAT CGC TCC AAG ACG CCA AAG AAC	1027
	Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys Thr Pro Lys Asn	
	295 300 305	
25	CAA GAG GCC CTG AGG ATG GCC AGT GTG GCA GAA AAC AGC AGC AGT GAC	1075
	Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn Ser Ser Ser Asp	
	310 315 320	
	CAG AGG CAG GCC TGC AAG AAA CAT GAG CTG TAC GTC AGC TTC CGA GAC	1123
	Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp	
	325 330 335 340	
30	CTT GGC TGG CAG GAC TGG ATC ATT GCA CCT GAA GGC TAT GCT GCC TAC	1171
	Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Tyr	
	345 350 355	
35	TAC TGT GAG GGA GAG TGC GCC TTC CCT CTG AAC TCC TAC ATG AAC GCC	1219
	Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser Tyr Met Asn Ala	
	360 365 370	
	ACC AAC CAC GCC ATC GTC CAG ACA CTG GTT CAC TTC ATC AAC CCA GAC	1267
	Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe Ile Asn Pro Asp	
	375 380 385	
40	ACA GTA CCC AAG CCC TGC TGT GCG CCC ACC CAG CTC AAC GCC ATC TCT	1315
	Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu Asn Ala Ile Ser	
	390 395 400	
45	GTC CTC TAC TTC GAC GAC AGC TCT AAT GTC ATC CTG AAG AAG TAC AGA	1363
	Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg	
	405 410 415 420	
50	AAC ATG GTG GTC CGG GCC TGT GGC TGC CAC TAGCTCTTCC TGAGACCCTG	1413
	Asn Met Val Val Arg Ala Cys Gly Cys His	
	425 430	
55	ACCTTTGCGG GGCCACACCT TTCCAAATCT TCGATGTCTC ACCATCTAAG TCTCTCACTG	1473
	CCCACCTTGG CGAGGAGAAC AGACCAACCT CTCCTGAGCC TTCCCTCACC TCCCAACCGG	1533
	AAGCATGTAA GGGTTCCAGA AACCTGAGCG TGCAGCAGCT GATGAGCGCC CTTTCCTTCT	1593
	GGCACGTGAC GGACAAGATC CTACCAGCTA CCACAGCAAA CGCCTAAGAG CAGGAAAAAT	1653

GTCTGCCAGG AAAGTGTCCA GTGTCCACAT GGCCCCTGGC GCTCTGAGTC TTTGAGGAGT 1713  
 AATCGCAAGC CTCGTTTCAGC TGCAGCAGAA GGAAGGGCTT AGCCAGGGTG GCGCTGGCG 1773  
 5 TCTGTGTTGA AGGGAAACCA AGCAGAAGCC ACTGTAATGA TATGTCACAA TAAAACCCAT 1833  
 GAATGAAAAA AAAAAAAAAA AAAAAAAAAA AAAAGAATTC 1873

10 (2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 430 amino acids

15 (B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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EP 0 812 207 B1

Met His Val Arg Ser Leu Arg Ala Ala Ala Pro His Ser Phe Val Ala  
1 5 10 15

5 Leu Trp Ala Pro Leu Phe Leu Leu Arg Ser Ala Leu Ala Asp Phe Ser  
20 25 30

Leu Asp Asn Glu Val His Ser Ser Phe Ile His Arg Arg Leu Arg Ser  
35 40 45

10 Gln Glu Arg Arg Glu Met Gln Arg Glu Ile Leu Ser Ile Leu Gly Leu  
50 55 60

Pro His Arg Pro Arg Pro His Leu Gln Gly Lys His Asn Ser Ala Pro  
65 70 75 80

15 Met Phe Met Leu Asp Leu Tyr Asn Ala Met Ala Val Glu Glu Ser Gly  
85 90 95

Pro Asp Gly Gln Gly Phe Ser Tyr Pro Tyr Lys Ala Val Phe Ser Thr  
100 105 110

20 Gln Gly Pro Pro Leu Ala Ser Leu Gln Asp Ser His Phe Leu Thr Asp  
115 120 125

Ala Asp Met Val Met Ser Phe Val Asn Leu Val Glu His Asp Lys Glu  
130 135 140

25 Phe Phe His Pro Arg Tyr His His Arg Glu Phe Arg Phe Asp Leu Ser  
145 150 155 160

30 Lys Ile Pro Glu Gly Glu Arg Val Thr Ala Ala Glu Phe Arg Ile Tyr  
165 170 175

Lys Asp Tyr Ile Arg Glu Arg Phe Asp Asn Glu Thr Phe Gln Ile Thr  
180 185 190

35 Val Tyr Gln Val Leu Gln Glu His Ser Gly Arg Glu Ser Asp Leu Phe  
195 200 205

40 Leu Leu Asp Ser Arg Thr Ile Trp Ala Ser Glu Glu Gly Trp Leu Val

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	210	215	220
5	Phe Asp Ile Thr Ala Thr Ser Asn His Trp Val Val Asn Pro Arg His 225 230 235 240		
	Asn Leu Gly Leu Gln Leu Ser Val Glu Thr Leu Asp Gly Gln Ser Ile 245 250 255		
10	Asn Pro Lys Leu Ala Gly Leu Ile Gly Arg His Gly Pro Gln Asn Lys 260 265 270		
	Gln Pro Phe Met Val Ala Phe Phe Lys Ala Thr Glu Val His Leu Arg 275 280 285		
15	Ser Ile Arg Ser Thr Gly Gly Lys Gln Arg Ser Gln Asn Arg Ser Lys 290 295 300		
	Thr Pro Lys Asn Gln Glu Ala Leu Arg Met Ala Ser Val Ala Glu Asn 305 310 315 320		
20	Ser Ser Ser Asp Gln Arg Gln Ala Cys Lys Lys His Glu Leu Tyr Val 325 330 335		
	Ser Phe Arg Asp Leu Gly Trp Gln Asp Trp Ile Ile Ala Pro Glu Gly 340 345 350		
25	Tyr Ala Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asn Ser 355 360 365		
	Tyr Met Asn Ala Thr Asn His Ala Ile Val Gln Thr Leu Val His Phe 370 375 380		
30	Ile Asn Pro Asp Thr Val Pro Lys Pro Cys Cys Ala Pro Thr Gln Leu 385 390 395 400		
	Asn Ala Ile Ser Val Leu Tyr Phe Asp Asp Ser Ser Asn Val Ile Leu 405 410 415		
35	Lys Lys Tyr Arg Asn Met Val Val Arg Ala Cys Gly Cys His 420 425 430		
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## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 1723 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

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- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: HIPPOCAMPUS

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 490..1695

(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"

/product= "hOP2-PP".

/note= "hOP2 (cDNA)"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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	GGCGCCGGCA GAGCAGGAGT GGCTGGAGGA GCTGTGGTTG GAGCAGGAGG TGGCACGGCA	60
	GGGCTGGAGG GCTCCCTATG AGTGGCGGAG ACGGCCAGG AGGCGCTGGA GCAACAGCTC	120
5	CCACACCGCA CCAAGCGGTG GCTGCAGGAG CTCGCCATC GCCCCTGCGC TGCTCGGACC	180
	GCGGCCACAG CCGGACTGGC GGGTACGGCG GCGACAGAGG CATTGGCCGA GAGTCCCAGT	240
10	CCGCAGAGTA GCCCCGGCCT CGAGGCGGTG GCGTCCCGGT CCTCTCCGTC CAGGAGCCAG	300
	GACAGGTGTC GCGCGGCGGG GCTCCAGGGA CCGCGCCTGA GGCCGGCTGC CCGCCCGTCC	360
	CGCCCCGCC CGCCGCCCGC CGCCGCCCGA GCCCAGCCTC CTTGCCGTCG GGGCGTCCCC	420
15	AGGCCCTGGG TCGGCCGCGG AGCCGATGCG CGCCCGCTGA GCGCCCCAGC TGAGCGCCCC	480
	CGGCCTGCC ATG ACC GCG CTC CCC GGC CCG CTC TGG CTC CTG GGC CTG	528
	Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu	
	1 5 10	
20	GCG CTA TGC GCG CTG GGC GGG GGC GGC CCC GGC CTG CGA CCC CCG CCC	576
	Ala Leu Cys Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro	
	15 20 25	
25	GGC TGT CCC CAG CGA CGT CTG GGC GCG CGC GAG CGC CGG GAC GTG CAG	624
	Gly Cys Pro Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln	
	30 35 40 45	
30	CGC GAG ATC CTG GCG GTG CTC GGG CTG CCT GGG CGG CCC CGG CCC CGC	672
	Arg Glu Ile Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg	
	50 55 60	
35	GCG CCA CCC GCC GCC TCC CGG CTG CCC GCG TCC GCG CCG CTC TTC ATG	720
	Ala Pro Pro Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met	
	65 70 75	
40	CTG GAC CTG TAC CAC GCC ATG GCC GGC GAC GAC GAC GAG GAC GGC GCG	768
	Leu Asp Leu Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala	
	80 85 90	
45	CCC GCG GAG CGG CGC CTG GGC CGC GCC GAC CTG GTC ATG AGC TTC GTT	816
	Pro Ala Glu Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val	
	95 100 105	
50	AAC ATG GTG GAG CGA GAC CGT GCC CTG GGC CAC CAG GAG CCC CAT TGG	864
	Asn Met Val Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp	
	110 115 120 125	
55	AAG GAG TTC CGC TTT GAC CTG ACC CAG ATC CCG GCT GGG GAG GCG GTC	912
	Lys Glu Phe Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val	
	130 135 140	
60	ACA GCT GCG GAG TTC CGG ATT TAC AAG GTG CCC AGC ATC CAC CTG CTC	960
	Thr Ala Ala Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu	
	145 150 155	
65	AAC AGG ACC CTC CAC GTC AGC ATG TTC CAG GTG GTC CAG GAG CAG TCC	1008
	Asn Arg Thr Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser	
	160 165 170	

5	AAC AGG GAG TCT GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA GCT Asn Arg Glu Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala 175 180 185	1056
10	GGA GAC GAG GGC TGG CTG GTG CTG GAT GTC ACA GCA GCC AGT GAC TGC Gly Asp Glu Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys 190 195 200 205	1104
15	TGG TTG CTG AAG CGT CAC AAG GAC CTG GGA CTC CGC CTC TAT GTG GAG Trp Leu Leu Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu 210 215 220	1152
20	ACT GAG GAC GGG CAC AGC GTG GAT CCT GGC CTG GCC GGC CTG CTG GGT Thr Glu Asp Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly 225 230 235	1200
25	CAA CGG GCC CCA CGC TCC CAA CAG CCT TTC GTG GTC ACT TTC TTC AGG Gln Arg Ala Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg 240 245 250	1248
30	GCC AGT CCG AGT CCC ATC CGC ACC CCT CGG GCA GTG AGG CCA CTG AGG Ala Ser Pro Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg 255 260 265	1296
35	AGG AGG CAG CCG AAG AAA AGC AAC GAG CTG CCG CAG GCC AAC CGA CTC Arg Arg Gln Pro Lys Lys Ser Asn Glu Leu Pro Gln Ala Asn Arg Leu 270 275 280 285	1344
40	CCA GGG ATC TTT GAT GAC GTC CAC GGC TCC CAC GGC CGG CAG GTC TGC Pro Gly Ile Phe Asp Asp Val His Gly Ser His Gly Arg Gln Val Cys 290 295 300	1392
45	CGT CGG CAC GAG CTC TAC GTC AGC TTC CAG GAC CTC GGC TGG CTG GAC Arg Arg His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Leu Asp 305 310 315	1440
50	TGG GTC ATC GCT CCC CAA GGC TAC TCG GCC TAT TAC TGT GAG GGG GAG Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu 320 325 330	1488
55	TGC TCC TTC CCA CTG GAC TCC TGC ATG AAT GCC ACC AAC CAC GCC ATC Cys Ser Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn His Ala Ile 335 340 345	1536
60	CTG CAG TCC CTG GTG CAC CTG ATG AAG CCA AAC GCA GTC CCC AAG GCG Leu Gln Ser Leu Val His Leu Met Lys Pro Asn Ala Val Pro Lys Ala 350 355 360 365	1584
65	TGC TGT GCA CCC ACC AAG CTG AGC GCC ACC TCT GTG CTC TAC TAT GAC Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp 370 375 380	1632
70	AGC AGC AAC AAC GTC ATC CTG CGC AAA CAC CGC AAC ATG GTG GTC AAG Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met Val Val Lys 385 390 395	1680
75	GCC TGC GGC TGC CAC TGAGTCAGCC CGCCAGCCCC TACTGCAG Ala Cys Gly Cys His 400	1723

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 402 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

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Met Thr Ala Leu Pro Gly Pro Leu Trp Leu Leu Gly Leu Ala Leu Cys  
1 5 10 15

Ala Leu Gly Gly Gly Gly Pro Gly Leu Arg Pro Pro Pro Gly Cys Pro  
20 25 30

Gln Arg Arg Leu Gly Ala Arg Glu Arg Arg Asp Val Gln Arg Glu Ile  
35 40 45

Leu Ala Val Leu Gly Leu Pro Gly Arg Pro Arg Pro Arg Ala Pro Pro  
50 55 60

Ala Ala Ser Arg Leu Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu  
65 70 75 80

Tyr His Ala Met Ala Gly Asp Asp Asp Glu Asp Gly Ala Pro Ala Glu  
85 90 95

Arg Arg Leu Gly Arg Ala Asp Leu Val Met Ser Phe Val Asn Met Val  
100 105 110

Glu Arg Asp Arg Ala Leu Gly His Gln Glu Pro His Trp Lys Glu Phe  
115 120 125

Arg Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala  
130 135 140

Glu Phe Arg Ile Tyr Lys Val Pro Ser Ile His Leu Leu Asn Arg Thr  
145 150 155 160

Leu His Val Ser Met Phe Gln Val Val Gln Glu Gln Ser Asn Arg Glu  
165 170 175

Ser Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ala Gly Asp Glu  
180 185 190

Gly Trp Leu Val Leu Asp Val Thr Ala Ala Ser Asp Cys Trp Leu Leu  
195 200 205

Lys Arg His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp  
210 215 220

Gly His Ser Val Asp Pro Gly Leu Ala Gly Leu Leu Gly Gln Arg Ala  
225 230 235 240

Pro Arg Ser Gln Gln Pro Phe Val Val Thr Phe Phe Arg Ala Ser Pro  
245 250 255

Ser Pro Ile Arg Thr Pro Arg Ala Val Arg Pro Leu Arg Arg Arg Gln  
260 265 270

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(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1926 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: MURIDAE  
(F) TISSUE TYPE: EMBRYO

(ix) **FEATURE:**

- (A) NAME/KEY: CDS  
(B) LOCATION: 93..1289  
(D) OTHER INFORMATION: /function= "OSTEOGENIC PROTEIN"  
/product= "mOP2-PP"  
/note= "mOP2 cDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:



	GCCAGGCACA GGTGCGCCGT CTGGTCCTCC CCGTCTGGCG TCAGCCGAGC CCGACCAGCT	60
5	ACCA GTGGAT GCGCGCCGCG TGAAAGTCCG AG ATG GCT ATG CGT CCC GGG CCA	113
	Met Ala Met Arg Pro Gly Pro	
	1 5	
10	CTC TGG CTA TTG GGC CTT GCT CTG TGC GCG CTG GGA GGC GGC CAC GGT	161
	Leu Trp Leu Leu Gly Leu Ala Leu Cys Ala Leu Gly Gly Gly His Gly	
	10 15 20	
15	CCG CGT CCC CCG CAC ACC TGT CCC CAG CGT CGC CTG GGA GCG CGC GAG	209
20		
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	Pro	Arg	Pro	Pro	His	Thr	Cys	Pro	Gln	Arg	Arg	Leu	Gly	Ala	Arg	Glu	
	25						30					35					
5	CGC	CGC	GAC	ATG	CAG	CGT	GAA	ATC	CTG	GCG	GTG	CTC	GGG	CTA	CCG	GGA	257
	Arg	Arg	Asp	Met	Gln	Arg	Glu	Ile	Leu	Ala	Val	Leu	Gly	Leu	Pro	Gly	
	40					45					50					55	
10	CGG	CCC	CGA	CCC	CGT	GCA	CAA	CCC	GCC	GCT	GCC	CGG	CAG	CCA	GCG	TCC	305
	Arg	Pro	Arg	Pro		Ala	Gln	Pro	Ala	Ala	Ala	Arg	Gln	Pro	Ala	Ser	
					60				65						70		
15	GCG	CCC	CTC	TTC	ATG	TTG	GAC	CTA	TAC	CAC	GCC	ATG	ACC	GAT	GAC	GAC	353
	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr	His	Ala	Met	Thr	Asp	Asp	Asp	
				75				80						85			
20	GAC	GGC	GGG	CCA	CCA	CAG	GCT	CAC	TTA	GGC	CGT	GCC	GAC	CTG	GTC	ATG	401
	Asp	Gly	Gly	Pro	Pro	Gln	Ala	His	Leu	Gly	Arg	Ala	Asp	Leu	Val	Met	
			90					95					100				
25	AGC	TTC	GTC	AAC	ATG	GTG	GAA	CGC	GAC	CGT	ACC	CTG	GGC	TAC	CAG	GAG	449
	Ser	Phe	Val	Asn	Met	Val	Glu	Arg	Asp	Arg	Thr	Leu	Gly	Tyr	Gln	Glu	
	105						110					115					
30	CCA	CAC	TGG	AAG	GAA	TTC	CAC	TTT	GAC	CTA	ACC	CAG	ATC	CCT	GCT	GGG	497
	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp	Leu	Thr	Gln	Ile	Pro	Ala	Gly	
	120					125					130					135	
35	GAG	GCT	GTC	ACA	GCT	GCT	GAG	TTC	CGG	ATC	TAC	AAA	GAA	CCC	AGC	ACC	545
	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	
					140					145					150		
40	CAC	CCG	CTC	AAC	ACA	ACC	CTC	CAC	ATC	AGC	ATG	TTC	GAA	GTG	GTC	CAA	593
	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile	Ser	Met	Phe	Glu	Val	Val	Gln	
				155					160					165			
45	GAG	CAC	TCC	AAC	AGG	GAG	TCT	GAC	TTG	TTC	TTT	TTG	GAT	CTT	CAG	ACG	641
	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu	Phe	Phe	Leu	Asp	Leu	Gln	Thr	
			170					175					180				
50	CTC	CGA	TCT	GGG	GAC	GAG	GGC	TGG	CTG	GTG	CTG	GAC	ATC	ACA	GCA	GCC	689
	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	Val	Leu	Asp	Ile	Thr	Ala	Ala	
		185					190					195					
55	AGT	GAC	CGA	TGG	CTG	CTG	AAC	CAT	CAC	AAG	GAC	CTG	GGA	CTC	CGC	CTC	737
	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	Lys	Asp	Leu	Gly	Leu	Arg	Leu	
	200					205					210					215	
60	TAT	GTG	GAA	ACC	GCG	GAT	GGG	CAC	AGC	ATG	GAT	CCT	GGC	CTG	GCT	GGT	785
	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	Met	Asp	Pro	Gly	Leu	Ala	Gly	
					220					225					230		
65	CTG	CTT	GGA	CGA	CAA	GCA	CCA	CGC	TCC	AGA	CAG	CCT	TTC	ATG	GTA	ACC	833
	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	Arg	Gln	Pro	Phe	Met	Val	Thr	
				235					240					245			
70	TTC	TTC	AGG	GCC	AGC	CAG	AGT	CCT	GTG	CGG	GCC	CCT	CGG	GCA	GCG	AGA	881
	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	Arg	Ala	Pro	Arg	Ala	Ala	Arg	
			250					255					260				
75	CCA	CTG	AAG	AGG	AGG	CAG	CCA	AAG	AAA	ACG	AAC	GAG	CTT	CCG	CAC	CCC	929
	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	Thr	Asn	Glu	Leu	Pro	His	Pro	
		265					270					275					

	AAC AAA CTC CCA GGG ATC TTT GAT GAT GGC CAC GGT TCC CGC GGC AGA	977
	Asn Lys Leu Pro Gly Ile Phe Asp Asp Gly His Gly Ser Arg Gly Arg	
	280 285 290 295	
5	GAG GTT TGC CGC AGG CAT GAG CTC TAC GTC AGC TTC CGT GAC CTT GGC	1025
	Glu Val Cys Arg Arg His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly	
	300 305 310	
10	TGG CTG GAC TGG GTC ATC GCC CCC CAG GGC TAC TCT GCC TAT TAC TGT	1073
	Trp Leu Asp Trp Val Ile Ala Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys	
	315 320 325	
15	GAG GGG GAG TGT GCT TTC CCA CTG GAC TCC TGT ATG AAC GCC ACC AAC	1121
	Glu Gly Glu Cys Ala Phe Pro Leu Asp Ser Cys Met Asn Ala Thr Asn	
	330 335 340	
20	CAT GCC ATC TTG CAG TCT CTG GTG CAC CTG ATG AAG CCA GAT GTT GTC	1169
	His Ala Ile Leu Gln Ser Leu Val His Leu Met Lys Pro Asp Val Val	
	345 350 355	
25	CCC AAG GCA TGC TGT GCA CCC ACC AAA CTG AGT GCC ACC TCT GTG CTG	1217
	Pro Lys Ala Cys Cys Ala Pro Thr Lys Leu Ser Ala Thr Ser Val Leu	
	360 365 370 375	
30	TAC TAT GAC AGC AGC AAC AAT GTC ATC CTG CGT AAA CAC CGT AAC ATG	1265
	Tyr Tyr Asp Ser Ser Asn Asn Val Ile Leu Arg Lys His Arg Asn Met	
	380 385 390	
35	GTG GTC AAG GCC TGT GGC TGC CAC TGAGGCCCG CCCAGCATCC TGCTTCTACT	1319
	Val Val Lys Ala Cys Gly Cys His	
	395	
40	ACCTTACCAT CTGGCCGGGC CCCTCTCCAG AGGCAGAAAC CCTTCTATGT TATCATAGCT	1379
45	CAGACAGGGG CAATGGGAGG CCCTTCACTT CCCCTGGCCA CTTCTCTGCTA AAATTCTGGT	1439
50	CTTTCCCACT TCCTCTGTCC TTCATGGGGT TTCGGGGCTA TCACCCCGCC CTCTCCATCC	1499
55	TCCTACCCCA AGCATAGACT GAATGCACAC AGCATCCCAG AGCTATGCTA ACTGAGAGGT	1559
60	CTGGGGTCAG CACTGAAGGC CCACATGAGG AAGACTGATC CTTGGCCATC CTCAGCCCAC	1619
65	AATGGCAAAT TCTGGATGGT CTAAGAAGGC CCTGGAATTC TAAACTAGAT GATCTGGGCT	1679
70	CTCTGCACCA TTCATTGTGG CAGTTGGGAC ATTTTGTAGGT ATAACAGACA CATACTTA	1739
75	GATCAATGCA TCGCTGTACT CCTTGAAATC AGAGCTAGCT TGTTAGAAAA AGAATCAGAG	1799
80	CCAGGTATAG CGGTGCATGT CATTAATCCC AGCGCTAAAG AGACAGAGAC AGGAGAATCT	1859
85	CTGTGAGTTC AAGGCCACAT AGAAAGAGCC TGTCTCGGGA GCAGGAAAAA AAAAAAAAC	1919
90	GGAATTC	1926

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 399 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

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	Met	Ala	Met	Arg	Pro	Gly	Pro	Leu	Trp	Leu	Leu	Gly	Leu	Ala	Leu	Cys	
	1				5					10					15		
5	Ala	Leu	Gly	Gly	Gly	His	Gly	Pro	Arg	Pro	Pro	His	Thr	Cys	Pro	Gln	
			20						25					30			
	Arg	Arg	Leu	Gly	Ala	Arg	Glu	Arg	Arg	Asp	Met	Gln	Arg	Glu	Ile	Leu	
			35					40					45				
10	Ala	Val	Leu	Gly	Leu	Pro	Gly	Arg	Pro	Arg	Pro	Arg	Ala	Gln	Pro	Ala	
		50					55					60					
	Ala	Ala	Arg	Gln	Pro	Ala	Ser	Ala	Pro	Leu	Phe	Met	Leu	Asp	Leu	Tyr	
15		65				70					75					80	
	His	Ala	Met	Thr	Asp	Asp	Asp	Asp	Gly	Gly	Pro	Pro	Gln	Ala	His	Leu	
					85					90					95		
	Gly	Arg	Ala	Asp	Leu	Val	Met	Ser	Phe	Val	Asn	Met	Val	Glu	Arg	Asp	
20				100						105				110			
	Arg	Thr	Leu	Gly	Tyr	Gln	Glu	Pro	His	Trp	Lys	Glu	Phe	His	Phe	Asp	
			115					120					125				
25	Leu	Thr	Gln	Ile	Pro	Ala	Gly	Glu	Ala	Val	Thr	Ala	Ala	Glu	Phe	Arg	
			130				135						140				
	Ile	Tyr	Lys	Glu	Pro	Ser	Thr	His	Pro	Leu	Asn	Thr	Thr	Leu	His	Ile	
		145				150					155				160		
30	Ser	Met	Phe	Glu	Val	Val	Gln	Glu	His	Ser	Asn	Arg	Glu	Ser	Asp	Leu	
					165					170					175		
	Phe	Phe	Leu	Asp	Leu	Gln	Thr	Leu	Arg	Ser	Gly	Asp	Glu	Gly	Trp	Leu	
35				180					185					190			
	Val	Leu	Asp	Ile	Thr	Ala	Ala	Ser	Asp	Arg	Trp	Leu	Leu	Asn	His	His	
			195					200						205			
	Lys	Asp	Leu	Gly	Leu	Arg	Leu	Tyr	Val	Glu	Thr	Ala	Asp	Gly	His	Ser	
40			210				215					220					
	Met	Asp	Pro	Gly	Leu	Ala	Gly	Leu	Leu	Gly	Arg	Gln	Ala	Pro	Arg	Ser	
		225				230					235					240	
45	Arg	Gln	Pro	Phe	Met	Val	Thr	Phe	Phe	Arg	Ala	Ser	Gln	Ser	Pro	Val	
					245					250					255		
	Arg	Ala	Pro	Arg	Ala	Ala	Arg	Pro	Leu	Lys	Arg	Arg	Gln	Pro	Lys	Lys	
					260				265					270			
50	Thr	Asn	Glu	Leu	Pro	His	Pro	Asn	Lys	Leu	Pro	Gly	Ile	Phe	Asp	Asp	
				275				280						285			
	Gly	His	Gly	Ser	Arg	Gly	Arg	Glu	Val	Cys	Arg	Arg	His	Glu	Leu	Tyr	
			290				295					300					
55	Val	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Leu	Asp	Trp	Val	Ile	Ala	Pro	Gln	

305                      310                      315                      320  
 Gly Tyr Ser Ala Tyr Tyr Cys Glu Gly Glu Cys Ala Phe Pro Leu Asp  
 5                                      325                                      330                                      335  
 Ser Cys Met Asn Ala Thr Asn His Ala Ile Leu Gln Ser Leu Val His  
 340                                      345                                      350  
 10                      Leu Met Lys Pro Asp Val Val Pro Lys Ala Cys Cys Ala Pro Thr Lys  
 355                                      360                                      365  
 Leu Ser Ala Thr Ser Val Leu Tyr Tyr Asp Ser Ser Asn Asn Val Ile  
 370                                      375                                      380  
 15                      Leu Arg Lys His Arg Asn Met Val Val Lys Ala Cys Gly Cys His  
 385                                      390                                      395

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1368 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1365
- (D) OTHER INFORMATION: /label= 60A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

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5	ATG TCG GGA CTG CGA AAC ACC TCG GAG GCC GTT GCA GTG CTC GCC TCC Met Ser Gly Leu Arg Asn Thr Ser Glu Ala Val Ala Val Leu Ala Ser 1 5 10 15	48
10	CTG GGA CTC GGA ATG GTT CTG CTC ATG TTC GTG GCG ACC ACG CCG CCG Leu Gly Leu Gly Met Val Leu Leu Met Phe Val Ala Thr Thr Pro Pro 20 25 30	96
15	GCC GTT GAG GCC ACC CAG TCG GGG ATT TAC ATA GAC AAC GGC AAG GAC Ala Val Glu Ala Thr Gln Ser Gly Ile Tyr Ile Asp Asn Gly Lys Asp 35 40 45	144
20	CAG ACG ATC ATG CAC AGA GTG CTG AGC GAG GAC GAC AAG CTG GAC GTC Gln Thr Ile Met His Arg Val Leu Ser Glu Asp Asp Lys Leu Asp Val 50 55 60	192
25	TCG TAC GAG ATC CTC GAG TTC CTG GGC ATC GCC GAA CGG CCG ACG CAC Ser Tyr Glu Ile Leu Glu Phe Leu Gly Ile Ala Glu Arg Pro Thr His 65 70 75 80	240
30	CTG AGC AGC CAC CAG TTG TCG CTG AGG AAG TCG GCT CCC AAG TTC CTG Leu Ser Ser His Gln Leu Ser Leu Arg Lys Ser Ala Pro Lys Phe Leu 85 90 95	288
35	CTG GAC GTC TAC CAC CGC ATC ACG GCG GAG GAG GGT CTC AGC GAT CAG	336

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	Leu	Asp	Val	Tyr	His	Arg	Ile	Thr	Ala	Glu	Glu	Gly	Leu	Ser	Asp	Gln	
				100					105					110			
5	GAT	GAG	GAC	GAC	GAC	TAC	GAA	CGC	GGC	CAT	CGG	TCC	AGG	AGG	AGC	GCC	384
	Asp	Glu	Asp	Asp	Asp	Tyr	Glu	Arg	Gly	His	Arg	Ser	Arg	Arg	Ser	Ala	
				115				120					125				
	GAC	CTC	GAG	GAG	GAT	GAG	GGC	GAG	CAG	CAG	AAG	AAC	TTC	ATC	ACC	GAC	432
10	Asp	Leu	Glu	Glu	Asp	Glu	Gly	Glu	Gln	Gln	Lys	Asn	Phe	Ile	Thr	Asp	
				130			135					140					
	CTG	GAC	AAG	CGG	GCC	ATC	GAC	GAG	AGC	GAC	ATC	ATC	ATG	ACC	TTC	CTG	480
	Leu	Asp	Lys	Arg	Ala	Ile	Asp	Glu	Ser	Asp	Ile	Ile	Met	Thr	Phe	Leu	
	145					150					155					160	
15	AAC	AAG	CGC	CAC	CAC	AAT	GTG	GAC	GAA	CTG	CGT	CAC	GAG	CAC	GGC	CGT	528
	Asn	Lys	Arg	His	His	Asn	Val	Asp	Glu	Leu	Arg	His	Glu	His	Gly	Arg	
				165				170							175		
20	CGC	CTG	TGG	TTC	GAC	GTC	TCC	AAC	GTG	CCC	AAC	GAC	AAC	TAC	CTG	GTG	576
	Arg	Leu	Trp	Phe	Asp	Val	Ser	Asn	Val	Pro	Asn	Asp	Asn	Tyr	Leu	Val	
				180				185						190			
	ATG	GCC	GAG	CTG	CGC	ATC	TAT	CAG	AAC	GCC	AAC	GAG	GGC	AAG	TGG	CTG	624
25	Met	Ala	Glu	Leu	Arg	Ile	Tyr	Gln	Asn	Ala	Asn	Glu	Gly	Lys	Trp	Leu	
				195				200					205				
	ACC	GCC	AAC	AGG	GAG	TTC	ACC	ATC	ACG	GTA	TAC	GCC	ATT	GGC	ACC	GGC	672
	Thr	Ala	Asn	Arg	Glu	Phe	Thr	Ile	Thr	Val	Tyr	Ala	Ile	Gly	Thr	Gly	
				210			215					220					
30	ACG	CTG	GGC	CAG	CAC	ACC	ATG	GAG	CCG	CTG	TCC	TCG	GTG	AAC	ACC	ACC	720
	Thr	Leu	Gly	Gln	His	Thr	Met	Glu	Pro	Leu	Ser	Ser	Val	Asn	Thr	Thr	
	225					230					235					240	
	GGG	GAC	TAC	GTG	GGC	TGG	TTG	GAG	CTC	AAC	GTG	ACC	GAG	GGC	CTG	CAC	768
35	Gly	Asp	Tyr	Val	Gly	Trp	Leu	Glu	Leu	Asn	Val	Thr	Glu	Gly	Leu	His	
				245				250						255			
	GAG	TGG	CTG	GTC	AAG	TCG	AAG	GAC	AAT	CAT	GGC	ATC	TAC	ATT	GGA	GCA	816
	Glu	Trp	Leu	Val	Lys	Ser	Lys	Asp	Asn	His	Gly	Ile	Tyr	Ile	Gly	Ala	
				260				265						270			
40	CAC	GCT	GTC	AAC	CGA	CCC	GAC	CGC	GAG	GTG	AAG	CTG	GAC	GAC	ATT	GGA	864
	His	Ala	Val	Asn	Arg	Pro	Asp	Arg	Glu	Val	Lys	Leu	Asp	Asp	Ile	Gly	
				275				280					285				
	CTG	ATC	CAC	CGC	AAG	GTG	GAC	GAC	GAG	TTC	CAG	CCC	TTC	ATG	ATC	GGC	912
45	Leu	Ile	His	Arg	Lys	Val	Asp	Asp	Glu	Phe	Gln	Pro	Phe	Met	Ile	Gly	
				290			295					300					
	TTC	TTC	CGC	GGA	CCG	GAG	CTG	ATC	AAG	GCG	ACG	GCC	CAC	AGC	AGC	CAC	960
50	Phe	Phe	Arg	Gly	Pro	Glu	Leu	Ile	Lys	Ala	Thr	Ala	His	Ser	Ser	His	
	305					310					315					320	
	CAC	AGG	AGC	AAG	CGA	AGC	GCC	AGC	CAT	CCA	CGC	AAG	CGC	AAG	AAG	TCG	1008
	His	Arg	Ser	Lys	Arg	Ser	Ala	Ser	His	Pro	Arg	Lys	Arg	Lys	Lys	Ser	
				325				330						335			
55	GTG	TCG	CCC	AAC	AAC	GTG	CCG	CTG	CTG	GAA	CCG	ATG	GAG	AGC	ACG	CGC	1056
	Val	Ser	Pro	Asn	Asn	Val	Pro	Leu	Leu	Glu	Pro	Met	Glu	Ser	Thr	Arg	
				340				345						350			



5 AGC TGC CAG ATG CAG ACC CTG TAC ATA GAC TTC AAG GAT CTG GGC TGG 1104  
 Ser Cys Gln Met Gln Thr Leu Tyr Ile Asp Phe Lys Asp Leu Gly Trp  
 355 360 365  
 10 CAT GAC TGG ATC ATC GCA CCA GAG GGC TAT GGC GCC TTC TAC TGC AGC 1152  
 His Asp Trp Ile Ile Ala Pro Glu Gly Tyr Gly Ala Phe Tyr Cys Ser  
 370 375 380  
 15 GGC GAG TGC AAT TTC CCG CTC AAT GCG CAC ATG AAC GCC ACG AAC CAT 1200  
 Gly Glu Cys Asn Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His  
 385 390 395 400  
 20 GCG ATC GTC CAG ACC CTG GTC CAC CTG CTG GAG CCC AAG AAG GTG CCC 1248  
 Ala Ile Val Gln Thr Leu Val His Leu Glu Pro Lys Lys Val Pro  
 405 410 415  
 25 AAG CCC TGC TGC GCT CCG ACC AGG CTG GGA GCA CTA CCC GTT CTG TAC 1296  
 Lys Pro Cys Cys Ala Pro Thr Arg Leu Gly Ala Leu Pro Val Leu Tyr  
 420 425 430  
 30 CAC CTG AAC GAC GAG AAT GTG AAC CTG AAA AAG TAT AGA AAC ATG ATT 1344  
 His Leu Asn Asp Glu Asn Val Asn Leu Lys Lys Tyr Arg Asn Met Ile  
 435 440 445  
 35 GTG AAA TCC TGC GGG TGC CAT TGA 1368  
 Val Lys Ser Cys Gly Cys His  
 450 455

30 (2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 455 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

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	Met	Ser	Gly	Leu	Arg	Asn	Thr	Ser	Glu	Ala	Val	Ala	Val	Leu	Ala	Ser	
	1					5				10					15		
5	Leu	Gly	Leu	Gly	Met	Val	Leu	Leu	Met	Phe	Val	Ala	Thr	Thr	Pro	Pro	
				20					25					30			
	Ala	Val	Glu	Ala	Thr	Gln	Ser	Gly	Ile	Tyr	Ile	Asp	Asn	Gly	Lys	Asp	
			35					40				45					
10	Gln	Thr	Ile	Met	His	Arg	Val	Leu	Ser	Glu	Asp	Asp	Lys	Leu	Asp	Val	
		50					55					60					
	Ser	Tyr	Glu	Ile	Leu	Glu	Phe	Leu	Gly	Ile	Ala	Glu	Arg	Pro	Thr	His	
	65					70					75					80	
15	Leu	Ser	Ser	His	Gln	Leu	Ser	Leu	Arg	Lys	Ser	Ala	Pro	Lys	Phe	Leu	
					85					90					95		
	Leu	Asp	Val	Tyr	His	Arg	Ile	Thr	Ala	Glu	Glu	Gly	Leu	Ser	Asp	Gln	
20				100					105						110		

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	Asp	Glu	Asp	Asp	Asp	Tyr	Glu	Arg	Gly	His	Arg	Ser	Arg	Arg	Ser	Ala	
			115					120					125				
5	Asp	Leu	Glu	Glu	Asp	Glu	Gly	Glu	Gln	Gln	Lys	Asn	Phe	Ile	Thr	Asp	
		130					135					140					
	Leu	Asp	Lys	Arg	Ala	Ile	Asp	Glu	Ser	Asp	Ile	Ile	Met	Thr	Phe	Leu	
		145				150					155					160	
10	Asn	Lys	Arg	His	His	Asn	Val	Asp	Glu	Leu	Arg	His	Glu	His	Gly	Arg	
				165						170					175		
	Arg	Leu	Trp	Phe	Asp	Val	Ser	Asn	Val	Pro	Asn	Asp	Asn	Tyr	Leu	Val	
				180					185					190			
15	Met	Ala	Glu	Leu	Arg	Ile	Tyr	Gln	Asn	Ala	Asn	Glu	Gly	Lys	Trp	Leu	
			195					200					205				
	Thr	Ala	Asn	Arg	Glu	Phe	Thr	Ile	Thr	Val	Tyr	Ala	Ile	Gly	Thr	Gly	
		210					215					220					
20	Thr	Leu	Gly	Gln	His	Thr	Met	Glu	Pro	Leu	Ser	Ser	Val	Asn	Thr	Thr	
		225				230					235					240	
	Gly	Asp	Tyr	Val	Gly	Trp	Leu	Glu	Leu	Asn	Val	Thr	Glu	Gly	Leu	His	
				245						250					255		
25	Glu	Trp	Leu	Val	Lys	Ser	Lys	Asp	Asn	His	Gly	Ile	Tyr	Ile	Gly	Ala	
			260					265						270			
	His	Ala	Val	Asn	Arg	Pro	Asp	Arg	Glu	Val	Lys	Leu	Asp	Asp	Ile	Gly	
30			275					280					285				
	Leu	Ile	His	Arg	Lys	Val	Asp	Asp	Glu	Phe	Gln	Pro	Phe	Met	Ile	Gly	
		290					295					300					
35	Phe	Phe	Arg	Gly	Pro	Glu	Leu	Ile	Lys	Ala	Thr	Ala	His	Ser	Ser	His	
		305				310					315					320	
	His	Arg	Ser	Lys	Arg	Ser	Ala	Ser	His	Pro	Arg	Lys	Arg	Lys	Lys	Ser	
				325						330					335		
40	Val	Ser	Pro	Asn	Asn	Val	Pro	Leu	Leu	Glu	Pro	Met	Glu	Ser	Thr	Arg	
				340					345					350			
	Ser	Cys	Gln	Met	Gln	Thr	Leu	Tyr	Ile	Asp	Phe	Lys	Asp	Leu	Gly	Trp	
			355					360					365				
45	His	Asp	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Gly	Ala	Phe	Tyr	Cys	Ser	
		370					375					380					
	Gly	Glu	Cys	Asn	Phe	Pro	Leu	Asn	Ala	His	Met	Asn	Ala	Thr	Asn	His	
		385				390					395					400	
50	Ala	Ile	Val	Gln	Thr	Leu	Val	His	Leu	Leu	Glu	Pro	Lys	Lys	Val	Pro	
				405						410					415		
	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Arg	Leu	Gly	Ala	Leu	Pro	Val	Leu	Tyr	
				420					425					430			
55	His	Leu	Asn	Asp	Glu	Asn	Val	Asn	Leu	Lys	Lys	Tyr	Arg	Asn	Met	Ile	
			435					440					445				

Val Lys Ser Cys Gly Cys His  
450 455

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(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 1674 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 69..1265
- (D) OTHER INFORMATION: /note= "mOP3-PP"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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	GGATCCGCGG CGCTGTCCCA TCCTTGTCGT CGAGGCGTCG CTGGATGCGA GTCCGCTAAA	60
5	CGTCCGAG ATG GCT GCG CGT CCG GGA CTC CTA TGG CTA CTG GGC CTG GCT Met Ala Ala Arg Pro Gly Leu Leu Trp Leu Leu Gly Leu Ala	110
	1 5 10	
10	CTG TGC GTG TTG GGC GGC GGT CAC CTC TCG CAT CCC CCG CAC GTC TTT Leu Cys Val Leu Gly Gly His Leu Ser His Pro Pro His Val Phe	158
	15 20 25 30	
	CCC CAG CGT CGA CTA GGA GTA CGC GAG CCC CGC GAC ATG CAG CGC GAG Pro Gln Arg Arg Leu Gly Val Arg Glu Pro Arg Asp Met Gln Arg Glu	206
	35 40 45	
15	ATT CGG GAG GTG CTG GGG CTA GCC GGG CGG CCC CGA TCC CGA GCA CCG Ile Arg Glu Val Leu Gly Leu Ala Gly Arg Pro Arg Ser Arg Ala Pro	254
	50 55 60	
20	GTC GGG GCT GCC CAG CAG CCA GCG TCT GCG CCC CTC TTT ATG TTG GAC Val Gly Ala Ala Gln Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp	302
	65 70 75	
25	CTG TAC CGT GCC ATG ACG GAT GAC AGT GGC GGT GGG ACC CCG CAG CCT Leu Tyr Arg Ala Met Thr Asp Asp Ser Gly Gly Gly Thr Pro Gln Pro	350
	80 85 90	
	CAC TTG GAC CGT GCT GAC CTG ATT ATG AGC TTT GTC AAC ATA GTG GAA His Leu Asp Arg Ala Asp Leu Ile Met Ser Phe Val Asn Ile Val Glu	398
	95 100 105 110	
30	CGC GAC CGT ACC CTG GGC TAC CAG GAG CCA CAC TGG AAG GAA TTC CAC Arg Asp Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His	446
	115 120 125	
35	TTT GAC CTA ACC CAG ATC CCT GCT GGG GAG GCT GTC ACA GCT GCT GAG Phe Asp Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu	494
	130 135 140	
40	TTC CGG ATC TAC AAA GAA CCC AGT ACC CAC CCG CTC AAC ACA ACC CTC Phe Arg Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu	542
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	145	150	155	
5	CAC ATC AGC ATG TTC GAA GTG GTC CAA GAG CAC TCC AAC AGG GAG TCT His Ile Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser 160. 165 170	590		
	GAC TTG TTC TTT TTG GAT CTT CAG ACG CTC CGA TCT GGG GAC GAG GGC Asp Leu Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly 175 180 185 190	638		
10	TGG CTG GTG CTG GAC ATC ACA GCA GCC AGT GAC CGA TGG CTG CTG AAC Trp Leu Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn 195 200 205	686		
15	CAT CAC AAG GAC CTA GGA CTC CGC CTC TAT GTG GAA ACC GAG GAT GGG His His Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly 210 215 220	734		
20	CAC AGC ATA GAT CCT GGC CTA GCT GGT CTG CTT GGA CGA CAA GCA CCA His Ser Ile Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro 225 230 235	782		
	CGC TCC AGA CAG CCT TTC ATG GTT GGT TTC TTC AGG GCC AAC CAG AGT Arg Ser Arg Gln Pro Phe Met Val Gly Phe Phe Arg Ala Asn Gln Ser 240 245 250	830		
25	CCT GTG CGG GCC CCT CGA ACA GCA AGA CCA CTG AAG AAG AAG CAG CTA Pro Val Arg Ala Pro Arg Thr Ala Arg Pro Leu Lys Lys Lys Gln Leu 255 260 265 270	878		
30	AAT CAA ATC AAC CAG CTG CCG CAC TCC AAC AAA CAC CTA GGA ATC CTT Asn Gln Ile Asn Gln Leu Pro His Ser Asn Lys His Leu Gly Ile Leu 275 280 285	926		
	GAT GAT GGC CAC GGT TCT CAC GGC AGA GAA GTT TGC CGC AGG CAT GAG Asp Asp Gly His Gly Ser His Gly Arg Glu Val Cys Arg Arg His Glu 290 295 300	974		
35	CTC TAT GTC AGC TTC CGT GAC CTT GGC TGG CTG GAC TCT GTC ATT GCC Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Ser Val Ile Ala 305 310 315	1022		
40	CCC CAG GGC TAC TCC GCC TAT TAC TGT GCT GGG GAG TGC ATC TAC CCA Pro Gln Gly Tyr Ser Ala Tyr Tyr Cys Ala Gly Glu Cys Ile Tyr Pro 320 325 330	1070		
	CTG AAC TCC TGT ATG AAC TCC ACC AAC CAC GCC ACT ATG CAG GCC CTG Leu Asn Ser Cys Met Asn Ser Thr Asn His Ala Thr Met Gln Ala Leu 335 340 345 350	1118		
45	GTA CAT CTG ATG AAG CCA GAT ATC ATC CCC AAG GTG TGC TGT GTG CCT Val His Leu Met Lys Pro Asp Ile Ile Pro Lys Val Cys Cys Val Pro 355 360 365	1166		
50	ACT GAG CTG AGT GCC ATT TCT CTG CTC TAC TAT GAT AGA AAC AAT AAT Thr Glu Leu Ser Ala Ile Ser Leu Leu Tyr Tyr Asp Arg Asn Asn Asn 370 375 380	1214		
	GTC ATC CTG CGC AGG GAG CGC AAC ATG GTA GTC CAG GCC TGT GGC TGC Val Ile Leu Arg Arg Glu Arg Asn Met Val Val Gln Ala Cys Gly Cys 385 390 395	1262		
55	CAC TGAGTCCCTG CCCAACAGCC TGCTGCCATC CCATCTATCT AGTCAGGCCT	1315		

His

5 CTCTTCCAAG GCAGGAAACC AACAAAGAGG GAAGGCAGTG CTTTCAACTC CATGTCCACA 1375  
 TTCACAGTCT TGGCCCTCTC TGTTCCTTTT GCCAAGGCTG AGAAGATGGT CCTAGTTATA 1435  
 ACCCTGGTGA C CTCAGTAGC CCGATCTCTC ATCTCCCCAA ACTCCCCAAT GCAGCCAGGG 1495  
 10 GCATCTATGT CTTTGGGAT TGGGCACAGA AGTCCAATTT ACCAACTTAT TCATGAGTCA 1555  
 CTACTGGCCC AGCCTGGACT TGAACCTGGA ACACAGGGTA GAGCTCAGGC TCTTCAGTAT 1615  
 CCATCAGAAG ATTTAGGTGT GTGCAGACAT GACCACACTC CCCCTAGCAC TCCATAGCC 1674  
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(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 399 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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Met Ala Ala Arg Pro Gly Leu Leu Trp Leu Leu Gly Leu Ala Leu Cys  
1 5 10 15

Val Leu Gly Gly Gly His Leu Ser His Pro Pro His Val Phe Pro Gln  
5 20 25 30

Arg Arg Leu Gly Val Arg Glu Pro Arg Asp Met Gln Arg Glu Ile Arg  
35 40 45

Glu Val Leu Gly Leu Ala Gly Arg Pro Arg Ser Arg Ala Pro Val Gly  
10 50 55 60

Ala Ala Gln Gln Pro Ala Ser Ala Pro Leu Phe Met Leu Asp Leu Tyr  
65 70 75 80

Arg Ala Met Thr Asp Asp Ser Gly Gly Gly Thr Pro Gln Pro His Leu  
15 85 90 95

Asp Arg Ala Asp Leu Ile Met Ser Phe Val Asn Ile Val Glu Arg Asp  
20 100 105 110

Arg Thr Leu Gly Tyr Gln Glu Pro His Trp Lys Glu Phe His Phe Asp  
115 120 125

Leu Thr Gln Ile Pro Ala Gly Glu Ala Val Thr Ala Ala Glu Phe Arg  
25 130 135 140

Ile Tyr Lys Glu Pro Ser Thr His Pro Leu Asn Thr Thr Leu His Ile  
145 150 155 160

Ser Met Phe Glu Val Val Gln Glu His Ser Asn Arg Glu Ser Asp Leu  
30 165 170 175

Phe Phe Leu Asp Leu Gln Thr Leu Arg Ser Gly Asp Glu Gly Trp Leu  
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	180	185	190
5	Val Leu Asp Ile Thr Ala Ala Ser Asp Arg Trp Leu Leu Asn His His 195 200 205		
	Lys Asp Leu Gly Leu Arg Leu Tyr Val Glu Thr Glu Asp Gly His Ser 210 215 220		
10	Ile Asp Pro Gly Leu Ala Gly Leu Leu Gly Arg Gln Ala Pro Arg Ser 225 230 235 240		
	Arg Gln Pro Phe Met Val Gly Phe Phe Arg Ala Asn Gln Ser Pro Val 245 250 255		
15	Arg Ala Pro Arg Thr Ala Arg Pro Leu Lys Lys Lys Gln Leu Asn Gln 260 265 270		
	Ile Asn Gln Leu Pro His Ser Asn Lys His Leu Gly Ile Leu Asp Asp 275 280 285		
20	Gly His Gly Ser His Gly Arg Glu Val Cys Arg Arg His Glu Leu Tyr 290 295 300		
	Val Ser Phe Arg Asp Leu Gly Trp Leu Asp Ser Val Ile Ala Pro Gln 305 310 315 320		
25	Gly Tyr Ser Ala Tyr Tyr Cys Ala Gly Glu Cys Ile Tyr Pro Leu Asn 325 330 335		
	Ser Cys Met Asn Ser Thr Asn His Ala Thr Met Gln Ala Leu Val His 340 345 350		
30	Leu Met Lys Pro Asp Ile Ile Pro Lys Val Cys Cys Val Pro Thr Glu 355 360 365		
	Leu Ser Ala Ile Ser Leu Leu Tyr Tyr Asp Arg Asn Asn Asn Val Ile 370 375 380		
35	Leu Arg Arg Glu Arg Asn Met Val Val Gln Ala Cys Gly Cys His 385 390 395		
40			

## (2) INFORMATION FOR SEQ ID NO:27:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..104
- (D) OTHER INFORMATION: /note= "BMP3"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27;

5 Cys Ala Arg Arg Tyr Leu Lys Val Asp Phe Ala Asp Ile Gly Trp Ser  
 1 5 10 15  
 10 Glu Trp Ile Ile Ser Pro Lys Ser Phe Asp Ala Tyr Tyr Cys Ser Gly  
 20 25 30  
 Ala Cys Gln Phe Pro Met Pro Lys Ser Leu Lys Pro Ser Asn His Ala  
 35 40 45  
 15 Thr Ile Gln Ser Ile Val Ala Arg Ala Val Gly Val Val Pro Gly Ile  
 50 55 60  
 Pro Glu Pro Cys Cys Val Pro Glu Lys Met Ser Ser Leu Ser Ile Leu  
 65 70 75 80  
 20 Phe Phe Asp Glu Asn Lys Asn Val Val Leu Lys Val Tyr Pro Asn Met  
 85 90 95  
 Thr Val Glu Ser Cys Ala Cys Arg  
 100

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: HOMO SAPIENS

40 (ix) FEATURE:

(A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /note= "BMP5"

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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Cys Lys Lys His Glu Leu Tyr Val Ser Phe Arg Asp Leu Gly Trp Gln  
 1 5 10 15  
 5 Asp Trp Ile Ile Ala Pro Glu Gly Tyr Ala Ala Phe Tyr Cys Asp Gly  
 20 25 30  
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 35 40 45  
 10 Ile Val Gln Thr Leu Val His Leu Met Phe Pro Asp His Val Pro Lys  
 50 55 60  
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
 65 70 75 80  
 15 Asp Asp Ser Ser Asn Val Ile Leu Lys Lys Tyr Arg Asn Met Val Val  
 85 90 95  
 Arg Ser Cys Gly Cys His  
 100

## (2) INFORMATION FOR SEQ ID NO:29:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 102 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS:  
 (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS

## (ix) FEATURE:

- (A) NAME/KEY: Protein  
 (B) LOCATION: 1..102  
 (D) OTHER INFORMATION: /note= "BMP6"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

1 Cys Arg Lys His Glu Leu Tyr Val Ser Phe Gln Asp Leu Gly Trp Gln  
 5 5 10 15  
 5 Asp Trp Ile Ile Ala Pro Lys Gly Tyr Ala Ala Asn Tyr Cys Asp Gly  
 20 25 30  
 Glu Cys Ser Phe Pro Leu Asn Ala His Met Asn Ala Thr Asn His Ala  
 35 40 45  
 10 Ile Val Gln Thr Leu Val His Leu Met Asn Pro Glu Tyr Val Pro Lys  
 50 55 60  
 Pro Cys Cys Ala Pro Thr Lys Leu Asn Ala Ile Ser Val Leu Tyr Phe  
 65 70 75 80  
 15 Asp Asp Asn Ser Asn Val Ile Leu Lys Lys Tyr Arg Trp Met Val Val  
 85 90 95  
 Arg Ala Cys Gly Cys His  
 20 100

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1247 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: HOMO SAPIENS
- (F) TISSUE TYPE: BRAIN

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 84..1199
- (D) OTHER INFORMATION: /product= "GDF-1"
- /note= "GDF-1 CDNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

	GGGGACACCG	GGCCCGCCCT	CAGCCCACTG	GTCCCGGGCC	GCCGCGGACC	CTGCGCACTC	60
	TCTGGTCATC	GCCTGGGAGG	AAG ATG CCA CCG CCG CAG CAA GGT CCC TGC	110			
5			Met Pro Pro Pro Gln Gln Gly Pro Cys	1	5		
	GGC CAC CAC CTC CTC CTC CTC CTG GCC CTG CTG CTG CCC TCG CTG CCC	158					
10	Gly His His Leu Leu Leu Leu Leu Ala Leu Leu Leu Pro Ser Leu Pro	10	15	20	25		
	CTG ACC CGC GCC CCC GTG CCC CCA GGC CCA GCC GCC GCC CTG CTC CAG	206					
	Leu Thr Arg Ala Pro Val Pro Pro Gly Pro Ala Ala Ala Leu Leu Gln	30	35	40			
15	GCT CTA GGA CTG CGC GAT GAG CCC CAG GGT GCC CCC AGG CTC CGG CCG	254					
	Ala Leu Gly Leu Arg Asp Glu Pro Gln Gly Ala Pro Arg Leu Arg Pro	45	50	55			
20	GTT CCC CCG GTC ATG TGG CGC CTG TTT CGA CGC CGG GAC CCC CAG GAG	302					
	Val Pro Pro Val Met Trp Arg Leu Phe Arg Arg Arg Asp Pro Gln Glu	60	65	70			
	ACC AGG TCT GGC TCG CGG CGG ACG TCC CCA GGG GTC ACC CTG CAA CCG	350					
25	Thr Arg Ser Gly Ser Arg Arg Thr Ser Pro Gly Val Thr Leu Gln Pro	75	80	85			
	TGC CAC GTG GAG GAG CTG GGG GTC GCC GGA AAC ATC GTG CGC CAC ATC	398					
	Cys His Val Glu Glu Leu Gly Val Ala Gly Asn Ile Val Arg His Ile	90	95	100	105		
30	CCG GAC CGC GGT GCG CCC ACC CGG GCC TCG GAG CCT GTC TCG GCC GCG	446					
	Pro Asp Arg Gly Ala Pro Thr Arg Ala Ser Glu Pro Val Ser Ala Ala	110	115	120			
35	GGG CAT TGC CCT GAG TGG ACA GTC GTC TTC GAC CTG TCG GCT GTG GAA	494					
	Gly His Cys Pro Glu Trp Thr Val Val Phe Asp Leu Ser Ala Val Glu	125	130	135			
	CCC GCT GAG CGC CCG AGC CGG GCC CGC CTG GAG CTG CGT TTC GCG GCG	542					
40	Pro Ala Glu Arg Pro Ser Arg Ala Arg Leu Glu Leu Arg Phe Ala Ala	140	145	150			
	GCG GCG GCG GCA GCC CCG GAG GGC GGC TGG GAG CTG AGC GTG GCG CAA	590					
	Ala Ala Ala Ala Ala Pro Glu Gly Gly Trp Glu Leu Ser Val Ala Gln	155	160	165			
45	GCG GGC CAG GGC GCG GGC GCG GAC CCC GGG CCG GTG CTG CTC CGC CAG	638					
	Ala Gly Gln Gly Ala Gly Ala Asp Pro Gly Pro Val Leu Leu Arg Gln	170	175	180	185		
50	TTG GTG CCC GCC CTG GGG CCG CCA GTG CGC GCG GAG CTG CTG GGC GCC	686					
	Leu Val Pro Ala Leu Gly Pro Pro Val Arg Ala Glu Leu Leu Gly Ala	190	195	200			
55	GCT TGG GCT CGC AAC GCC TCA TGG CCG CGC AGC CTC CGC CTG GCG CTG	734					
	Ala Trp Ala Arg Asn Ala Ser Trp Pro Arg Ser Leu Arg Leu Ala Leu						

	205	210	215	
5	GCG CTA CGC CCC CGG GCC CCT GCC GCC TGC GCG CGC CTG GCC GAG GCC Ala Leu Arg Pro Arg Ala Pro Ala Ala Cys Ala Arg Leu Ala Glu Ala 220 225 230			782
10	TCG CTG CTG CTG GTG ACC CTC GAC CCG CGC CTG TGC CAC CCC CTG GCC Ser Leu Leu Leu Val Thr Leu Asp Pro Arg Leu Cys His Pro Leu Ala 235 240 245			830
15	CGG CCG CGG CGC GAC GCC GAA CCC GTG TTG GGC GGC GGC CCC GGG GGC Arg Pro Arg Arg Asp Ala Glu Pro Val Leu Gly Gly Gly Pro Gly Gly 250 255 260 265			878
20	GCT TGT CGC GCG CGG CGG CTG TAC GTG AGC TTC CGC GAG GTG GGC TGG Ala Cys Arg Ala Arg Arg Leu Tyr Val Ser Phe Arg Glu Val Gly Trp 270 275 280			926
25	CAC CGC TGG GTC ATC GCG CCG CGC GGC TTC CTG GCC AAC TAC TGC CAG His Arg Trp Val Ile Ala Pro Arg Gly Phe Leu Ala Asn Tyr Cys Gln 285 290 295			974
30	GGT CAG TGC GCG CTG CCC GTC GCG CTG TCG GGG TCC GGG GGG CCG CCG Gly Gln Cys Ala Leu Pro Val Ala Leu Ser Gly Ser Gly Gly Pro Pro 300 305 310			1022
35	GCG CTC AAC CAC GCT GTG CTG CGC GCG CTC ATG CAC GCG GCC GCC CCG Ala Leu Asn His Ala Val Leu Arg Ala Leu Met His Ala Ala Ala Pro 315 320 325			1070
40	GGA GCC GCC GAC CTG CCC TGC TGC GTG CCC GCG CGC CTG TCG CCC ATC Gly Ala Ala Asp Leu Pro Cys Cys Val Pro Ala Arg Leu Ser Pro Ile 330 335 340 345			1118
45	TCC GTG CTC TTC TTT GAC AAC AGC GAC AAC GTG GTG CTG CGG CAG TAT Ser Val Leu Phe Phe Asp Asn Ser Asp Asn Val Val Leu Arg Gln Tyr 350 355 360			1166
50	GAG GAC ATG GTG GTG GAC GAG TGC GGC TGC CGC TAACCCGGGG CGGGCAGGGA Glu Asp Met Val Val Asp Glu Cys Gly Cys Arg 365 370			1219
55	CCCGGGCCCA ACAATAAATG CCGCGTGG			1247

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 372 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

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Met Pro Pro Pro Gln Gln Gly Pro Cys Gly His His Leu Leu Leu Leu  
 1 5 10 15

Leu Ala Leu Leu Leu Pro Ser Leu Pro Leu Thr Arg Ala Pro Val Pro  
 20 25 30

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	Pro Gly	Pro Ala	Ala Ala	Ala Leu	Leu Leu	Gln Ala	Leu Gly	Leu Arg	Asp Glu	
	35				40			45		
5	Pro Gln	Gly Ala	Pro Arg	Leu Arg	Pro Val	Pro Pro	Val Met	Trp Arg		
	50			55			60			
	Leu Phe	Arg Arg	Arg Asp	Pro Gln	Glu Thr	Arg Ser	Gly Ser	Arg Arg		
	65		70			75		80		
10	Thr Ser	Pro Gly	Val Thr	Leu Gln	Pro Cys	His Val	Glu Glu	Leu Gly		
			85		90			95		
	Val Ala	Gly Asn	Ile Val	Arg His	Ile Pro	Asp Arg	Gly Ala	Pro Thr		
		100			105		110			
15	Arg Ala	Ser Glu	Pro Val	Ser Ala	Ala Gly	His Cys	Pro Glu	Trp Thr		
		115			120		125			
	Val Val	Phe Asp	Leu Ser	Ala Val	Glu Pro	Ala Glu	Arg Pro	Ser Arg		
	130			135		140				
20	Ala Arg	Leu Glu	Leu Arg	Phe Ala	Ala Ala	Ala Ala	Ala Ala	Pro Glu		
	145		150			155		160		
	Gly Gly	Trp Glu	Leu Ser	Val Ala	Gln Ala	Gly Gln	Gly Ala	Gly Ala		
			165		170			175		
25	Asp Pro	Gly Pro	Val Leu	Leu Arg	Gln Leu	Val Pro	Ala Leu	Gly Pro		
		180			185		190			
	Pro Val	Arg Ala	Glu Leu	Leu Gly	Ala Ala	Trp Ala	Arg Asn	Ala Ser		
		195			200		205			
30	Trp Pro	Arg Ser	Leu Arg	Leu Ala	Leu Ala	Leu Arg	Pro Arg	Ala Pro		
	210			215		220				
	Ala Ala	Cys Ala	Arg Leu	Ala Glu	Ala Ser	Leu Leu	Leu Val	Thr Leu		
35	225		230			235		240		
	Asp Pro	Arg Leu	Cys His	Pro Leu	Ala Arg	Pro Arg	Arg Asp	Ala Glu		
			245		250			255		
40	Pro Val	Leu Gly	Gly Gly	Pro Gly	Gly Ala	Cys Arg	Ala Arg	Arg Leu		
		260			265		270			
	Tyr Val	Ser Phe	Arg Glu	Val Gly	Trp His	Arg Trp	Val Ile	Ala Pro		
		275			280		285			
45	Arg Gly	Phe Leu	Ala Asn	Tyr Cys	Gln Gly	Gln Cys	Ala Leu	Pro Val		
	290			295		300				
	Ala Leu	Ser Gly	Ser Gly	Gly Gly	Pro Pro	Ala Leu	Asn His	Ala Val	Leu	
	305			310		315			320	
50	Arg Ala	Leu Met	His Ala	Ala Ala	Pro Gly	Ala Ala	Asp Leu	Pro Cys		
			325		330			335		
	Cys Val	Pro Ala	Arg Leu	Ser Pro	Ile Ser	Val Leu	Phe Phe	Asp Asn		
		340			345		350			
55	Ser Asp	Asn Val	Val Leu	Arg Gln	Tyr Glu	Asp Met	Val Val	Asp Glu		
		355			360		365			



Cys Gly Cys Arg  
370

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(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

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- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS:
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Arg Xaa Xaa Arg  
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### Claims

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1. Use of a morphogen for the manufacture of a medicament for use in:

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- (a) stimulating morphogenesis of dentine in a mammalian tooth; or
- (b) stimulating phenotypic expression of mammalian odontoblasts; or
- (c) stimulating production of dentine matrix by mammalian odontoblasts; or
- (d) increasing thickness of a mammalian tooth wall; or
- (e) reducing risk of fracture in a mammalian tooth; or
- (f) desensitizing a mammalian tooth to perception of pressure or temperature; or
- (g) sealing a cavity in a mammalian tooth; wherein said medicament is applied to a dentinal surface.

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2. The use of claim 1 wherein the dentinal surface either: (i) adjoins a site of lost or damaged enamel, dentine or cementum tissue, such as a cavity, of said tooth, or (ii) adjoins a site of lost or damaged gingival tissue.

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3. The use of claim 1 or claim 2 wherein the dentinal surface has been treated either to:- (i) ablate damaged or infected enamel, dentine or cementum tissue from the site of said cavity, or (ii) debride damaged gingival, enamel, dentine or cementum tissue from said dentinal surface.

4. The use of any one of claims 1 to 3 comprising the application of said morphogen in an amount effective for stimulating formation of reparative dentine apposite said dentinal surface.

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5. The use of claim 4 wherein said dentinal surface is transverse to luminae of dental canaliculi within said tooth.

6. The use of any one of the preceding claims wherein said dentinal surface is separated from the pulp chamber wall of said tooth by up to about 1 mm of residual dentine.

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7. The use of any one of the preceding claims wherein:

- (A) said morphogen is solubilised in a physiologically acceptable vehicle or an evaporative vehicle; or
- (B) said morphogen is adsorbed on a biocompatible, acellular matrix suitable for sealing or filling defects in mammalian teeth.

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8. The use of claim 7(A) wherein the morphogen is solubilized by the preparative step of solubilising said morphogen in a physiologically acceptable vehicle or an evaporative vehicle, said vehicle optionally further comprising a co-factor that mitigates symptoms associated with tooth damage.

9. The use of claim 7(B) wherein the morphogen is adsorbed by the preparative step of adsorbing said morphogen on a biocompatible, acellular matrix suitable for sealing or filling defects in mammalian teeth, said matrix optionally further comprising a cofactor that mitigates symptoms associated with tooth damage.
10. The use of any one of the preceding claims wherein said morphogen comprises a dimeric protein that induces morphogenesis of mammalian dentine tissue, said dimeric protein comprising a pair of folded polypeptides, the amino acid sequence of each of which comprises
- (i) a sequence sharing at least 70% homology with the C-terminal seven cysteine domain of human OP1, residues 38-139 of Seq. ID No. 4;
  - (ii) a sequence encoded by a nucleic acid that hybridizes under stringent conditions with nucleic acid encoding said domain of human OP1; or
  - (iii) a sequence defined by Generic Sequence 8, Seq. ID No. 2.
11. The use of claim 10 wherein: (i) said sequence of said morphogen polypeptides is defined by OPX, Seq. ID No. 3 and/or (ii) the morphogen is obtained from culture medium of morphogen-secreting mammalian cells.
12. The use of claim 10 or 11 wherein said sequence of said morphogen polypeptides is selected independently in each said polypeptide from the sequences of the C-terminal seven cysteine domains of human OP1, mouse OP1, human OP2, mouse OP2, mouse OP3, Drosophila 60A protein, Xenopus Vgl, mouse Vgr-1, mouse GDF-1, Drosophila DPP, CBMP2A, CBMP2B, BMP3, BMP5, BMP6 (shown in Seq. ID Nos. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 24, 26, 27, 28 and 29), and allelic, phylogenetic and biosynthetic variants thereof.
13. The use of claim 10 or 11 wherein said sequence of said morphogen polypeptides is selected, in each said polypeptide, from the sequences of the C-terminal seven cysteine domains of human OP-1, human OP-2, mouse OP-1, mouse OP-2, mouse OP-3 and Drosophila 60A protein (shown in seq. ID Nos. 4, 5, 6, 7, 24 and 26), and allelic, phylogenetic and biosynthetic variants thereof.
14. The use of claim 10 or 11 wherein said morphogen is solubilised by association with at least one morphogen prodomain polypeptide or solubility-enhancing fragment thereof.

#### Patentansprüche

1. Verwendung eines Morphogens zur Herstellung eines Medikamentes zur Verwendung bei:
- (a) Stimulieren der Dentinmorphogenese in einem Säugetierzahn; oder
  - (b) Stimulieren der phänotypischen Expression von Säugetier- Odontoblasten; oder
  - (c) Stimulieren der Erzeugung von Dentinmatrix durch Säugetier- Odontoblasten; oder
  - (d) Erhöhen der Dicke eines Säugetierzahnes; oder
  - (e) Vermindern des Bruchrisikos bei einem Säugetierzahn; oder
  - (f) Desensibilisieren eines Säugetierzahns gegenüber dem Wahrnehmungsvermögen von Druck oder Temperatur; oder
  - (g) Versiegeln einer Höhle in einem Säugetierzahn; wobei das Medikament auf eine Dentinal-Oberfläche aufgebracht wird.
2. Verwendung nach Anspruch 1, bei der die Dentinal-Oberfläche entweder: (i) an eine Stelle verlorenen oder beschädigten Zahnschmelzes, Dentin- oder Zementgewebes, wie beispielsweise einer Höhle dieses Zahnes, angrenzt oder (ii) an eine Stelle eines verlorenen oder beschädigten Zahnfleisch-Gewebes angrenzt.
3. Verwendung nach Anspruch 1 oder Anspruch 2, bei der die Dentinal-Oberfläche entweder zum: (i) Abtragen von beschädigtem oder infiziertem Zahnschmelz, Dentin- oder Zementgewebe von der Stelle dieser Kavität oder zum (ii) Reinigen von geschädigtem Zahnfleisch-, Zahnschmelz-, Dentin- oder Zementgewebe von dieser Dentinal-Oberfläche behandelt wird.
4. Verwendung nach einem der Ansprüche 1-3, die die Anwendung des Morphogens in einer zur Stimulierung der Bildung von Reparaturdentin wirksamen Menge umfaßt, die der Dentinal-Oberfläche angemessen ist.

5. Verwendung nach Anspruch 4, bei der die Dentinal-Oberfläche quer zu Hohlräumen von Dentalkanälchen innerhalb des Zahns verläuft.
6. Verwendung nach einem der vorhergehenden Ansprüche, bei der die Dentinal- Oberfläche von der Pulpakammerwand des Zahns von bis zu ungefähr 1 Millimeter Restdentin getrennt ist.
7. Verwendung nach einem der vorhergehenden Ansprüche, bei der:
  - (A) Das Morphogen in einem physiologisch akzeptablen Träger oder einem Verdunstungsträger solubilisiert ist; oder
  - (B) das Morphogen an eine biokompatible, azelluläre Matrix adsorbiert ist, die zum Versiegeln oder Füllen von Defekten in Säugetierzähnen geeignet ist.
8. Verwendung nach Anspruch 7A, bei der das Morphogen durch den Vorbereitungsschritt, das Morphogen in einem physiologisch akzeptablen Träger oder in einem Verdunstungsträger zu solubilisieren, solubilisiert wird, wobei der Träger optional weiterhin einen Co-Faktor umfaßt, der mit Zahnbeschädigung verbundene Symptome lindert.
9. Verwendung nach Anspruch 7B, bei der das Morphogen durch den Vorbereitungsschritt des Adsorbierens des Morphogens an eine biokompatible, azelluläre Matrix adsorbiert wird, die zum Versiegeln und Füllen von Defekten in Säugetierzähnen geeignet ist, wobei die Matrix optional weiterhin einen Co-Faktor umfaßt, der mit einer Zahnbeschädigung verbundene Symptome lindert.
10. Verwendung nach einem der vorhergehenden Ansprüche, bei der das Morphogen ein dimeres Protein umfaßt, das die Morphogenese von Säugetier-Dentingewebe induziert, wobei das dimere Protein zwei gefaltete Polypeptide umfaßt, deren Aminosäuresequenz jeweils
  - (i) eine Sequenz, die zumindest 70% Homologie mit der C-terminalen 7-Cystein-Domäne von menschlichem OP1, Reste 38 bis 139 von Sequenz ID Nr. 4, teilt;
  - (ii) eine Sequenz, die von einer Nukleinsäure kodiert wird, die unter stringenten Bedingungen mit einer Nukleinsäure hybridisiert, die diese Domäne von menschlichem OP1 kodiert; oder
  - (iii) eine Sequenz umfaßt, die durch Gattungssequenz 8, Sequenz ID-Nr. 2 definiert ist.
11. Verwendung nach Anspruch 10, bei der: (i) die Sequenz dieser Morphogen- Polypeptide durch OPX, Sequenz ID-Nr. 3 definiert ist und/oder (ii) das Morphogen aus Kulturmedium Morphogen-sezernierender Säugetierzellen gewonnen wird.
12. Verwendung nach Anspruch 10 oder 11, bei der die Sequenz des Morphogen- Polypeptids unabhängig bei jedem dieser Polypeptide aus den Sequenzen der C-Terminalen 7-Cystein-Domänen von menschlichem OP1, Maus-OP1, menschlichem OP2, Maus-OP2, Maus-OP3, Drosophila 60A-Protein, Xenopus Vgl, Maus Vgr-1, Maus-GDF-1, Drosophila-DPP, CBMP2A, CBMP2B, BMP3, BMP5, BMP6 (dargestellt in den Sequenz ID-Nr. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 24, 26, 27, 28 und 29) und Allel-, phylogenetische und biosynthetische Varianten hiervon, ausgewählt ist.
13. Verwendung nach Anspruch 10 oder 11, bei der die Sequenz des Morphogen- Polypeptids bei jedem Polypeptid aus den Sequenzen der C-Terminalen 7-Zystein-Domänen von menschlichem OP1, menschlichem OP2, Maus-OP1, Maus-OP2, Maus-OP3 und Drosophila 60A-Protein (dargestellt in den Sequenz ID-Nr. 4, 5, 6, 7, 24 und 26) und Allel-, phylogenetischen und biosynthetischen Varianten hiervon, ausgewählt ist.
14. Verwendung nach Anspruch 10 oder 11, bei der das Morphogen durch Verbindung mit zumindest einem Morphogen-Prodomän-Polypeptid oder Löslichkeits-verbessernden Bruchstücks hiervon solubilisiert wird.

## Revendications

1. Utilisation d'un morphogène pour la fabrication d'un médicament pour une utilisation :

- (a) pour la stimulation de la morphogenèse de la dentine de la dent d'un mammifère; ou  
 (b) pour la stimulation de l'expression phénotypique des odontoblastes de mammifères; ou  
 (c) pour la stimulation de la production de la matrice de la dentine par des odontoblastes de mammifères; ou  
 (d) pour l'augmentation de l'épaisseur de la paroi d'une dent de mammifère; ou  
 (e) pour la réduction du risque de fracture d'une dent de mammifère; ou  
 (f) pour la désensibilisation de la dent d'un mammifère à la perception d'une pression ou d'une température; ou  
 (g) pour le scellage d'une cavité d'une dent de mammifère;
- au cours de laquelle le dit médicament est appliqué sur une surface dentinale.
2. Utilisation selon la revendication 1 au cours de laquelle la surface dentinale soit: (i) est contiguë à un site de perte ou d'endommagement de l'émail, de la dentine ou d'un tissu du ciment, tel qu'une cavité de cette dent soit (ii) est contiguë à un site de perte ou d'endommagement d'un tissu gingival.
  3. Utilisation selon la revendication 1 ou 2 au cours de laquelle la surface dentinale a été traitée soit pour: - (i) pratiquer l'ablation d'un émail, d'une dentine, ou d'un tissu du ciment endommagé ou infecté à partir du site de cette cavité soit (ii) débrider l'émail, la dentine ou le tissu du ciment gingival endommagé à partir de cette surface dentinale.
  4. Utilisation selon l'une quelconque des revendications 1 à 3 comprenant l'application de ce morphogène en une quantité efficace pour stimuler la formation de la dentine réparatrice imposée à cette surface dentinale.
  5. Utilisation selon la revendication 4 au cours de laquelle cette surface dentinale est transversale par rapport aux lumières des canalicules dentaires à l'intérieur de cette dent.
  6. Utilisation selon l'une quelconque des revendications précédentes au cours de laquelle cette surface dentinaire est séparée de la paroi de la chambre de la pulpe de cette dent par jusqu'à environ 1 mm de dentine résiduelle.
  7. Utilisation selon l'une quelconque des revendications précédentes au cours de laquelle:
    - (A) le dit morphogène est solubilisé dans un véhicule physiologiquement acceptable ou un véhicule évaporateur; ou
    - (B) le dit morphogène est adsorbé sur une matrice acellulaire, biocompatible, appropriée pour fermer ou combler des défauts dans les dents de mammifères.
  8. Utilisation selon la revendication 7 (A) au cours de laquelle le morphogène est solubilisé par l'étape préparatoire de solubilisation de ce morphogène dans un véhicule physiologiquement acceptable ou un véhicule évaporateur, ce véhicule comprenant en outre facultativement un cofacteur qui mitige les symptômes associés à un dommage d'une dent.
  9. Utilisation selon la revendication 7 (B) au cours de laquelle le morphogène est adsorbé par l'étape préparatoire d'adsorption de ce morphogène sur une matrice acellulaire biocompatible, appropriée pour fermer ou combler des défauts dans les dents des mammifères, cette matrice comprenant en outre un cofacteur qui mitige les symptômes associés à un dommage d'une dent.
  10. Utilisation selon l'une quelconque des revendications précédentes au cours de laquelle ce morphogène comprend une protéine dimère qui induit la morphogenèse d'un tissu de la dentine d'un mammifère, cette protéine dimère contenant une paire de polypeptides repliés, dont la séquence d'acides aminés de chacune d'elles comprend
    - (i) une séquence partageant au moins 70 % d'homologie avec le domaine C-terminal à sept cystéines de l'OP1 humaine, les résidus 38-139 de la séquence ID No. 4;
    - (ii) une séquence codée par un acide nucléique qui s'hybride sous des conditions stringentes avec un acide nucléique codant pour ce domaine de l'OP1 humaine; ou
    - (iii) une séquence définie par la séquence générique 8, Seq. ID No. 2.
  11. Utilisation selon la revendication 10 au cours de laquelle: (i) cette séquence de ces polypeptides morphogènes est définie par OPX, Seq. ID No. 3 et/ou (ii) on obtient le morphogène à partir d'un milieu de culture de cellules de mammifère sécrétant un morphogène.

- 5 12. Utilisation selon la revendication 10 ou 11 au cours de laquelle cette séquence de ces polypeptides morphogènes est choisie indépendamment dans chacun de ces polypeptides provenant des séquences des domaines C-terminaux à sept cystéines de l'OP1 humaine, OP1 de souris, OP2 humaine, OP2 de souris, OP3 de souris, protéine 60A de la drosophile, Vgl de xénope, Vgr-1 de souris, GDF-1 de souris, DPP de drosophile, CBMP2A, CBMP2B, BMP3, BMP5, BMP6 (montrés dans les Seq. ID No. 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 24, 26, 27, 28 et 29); des variants allèles, phylogénétiques et biosynthétiques de ceux-ci.
- 10 13. Utilisation selon la revendication 10 ou 11 au cours de laquelle cette séquence de ces polypeptides morphogènes est choisie, dans chacun de ces polypeptides, à partir des séquences des domaines C-terminaux à sept cystéines de l'OP1 humaine, OP2 humaine, OP1 de souris, OP2 de souris, OP3 de souris et de la protéine 60A de la drosophile (montrés dans les Seq. ID No. 4, 5, 6, 7, 24 et 26), et les variants allèles, phylogénétiques et biosynthétiques de ceux-ci.
- 15 14. Utilisation selon la revendication 10 ou 11 dans laquelle le dit morphogène est solubilisé par association avec au moins un polypeptide du prodomaine du morphogène ou du fragment d'activation de la solubilité de celui-ci.

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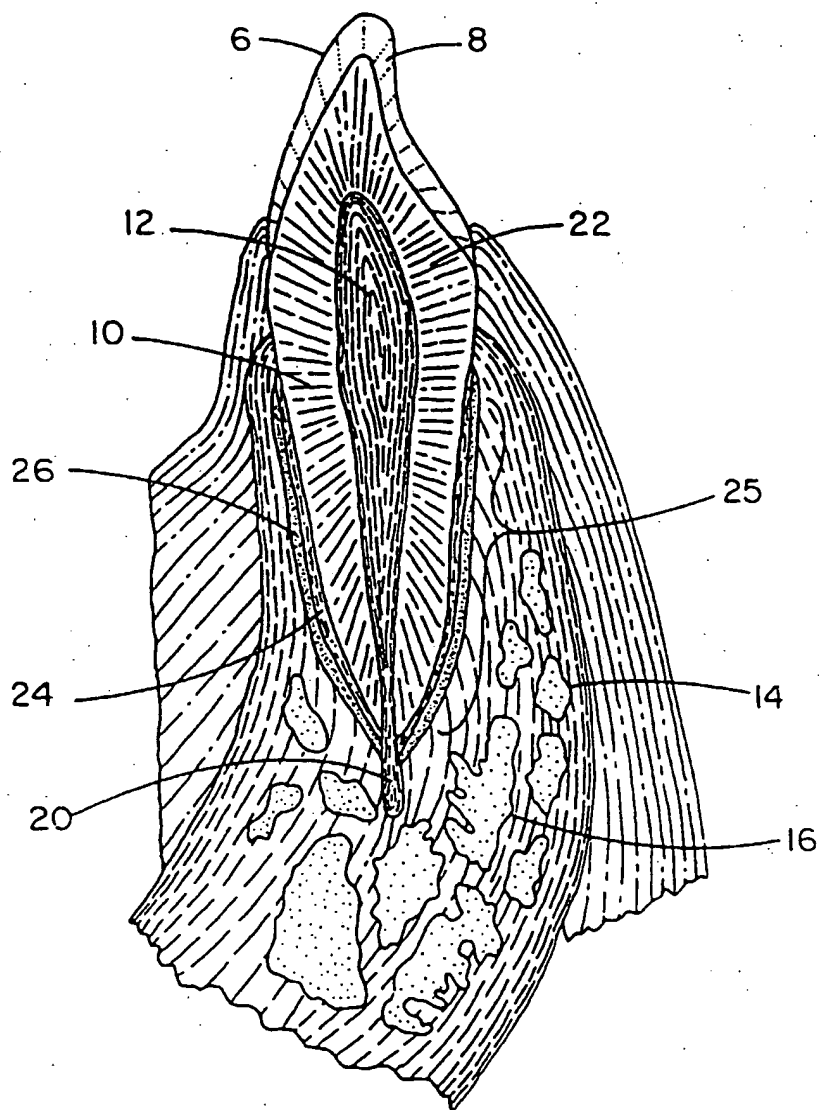
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*Fig.1*

	Cys	Lys	Lys	His	Glu	Leu	Tyr	Val
hOP-1	...	...	...	...	...	...	...	...
mOP-1	...	Arg	Arg	...	...	...	...	...
hOP-2	...	Arg	Arg	...	...	...	...	...
mOP-2	...	Arg	Arg	...	...	...	...	...
mOP-3	...	Arg	Arg	...	...	...	...	...
DPP	...	Arg	Arg	...	Ser	...	...	...
Vgl	...	...	Lys	Arg	His	...	...	...
Vgr-1	...	...	...	...	Gly	...	...	...
CBMP-2A	...	...	Arg	...	Pro	...	...	...
CBMP-2B	...	Arg	Arg	...	Ser	...	...	...
BMP3	...	Ala	Arg	Arg	Tyr	...	Lys	...
GDF-1	...	Arg	Ala	Arg	Arg	...	...	...
60A	...	Gln	Met	Glu	Thr	...	...	...
BMP5	...	...	...	...	...	...	...	...
BMP6	...	Arg	...	...	...	...	...	...
	1				5			

**FIGURE 2-1**

	Ser	Phe	Arg	Asp	Leu	Gly	Trp	Gln	Asp
hOP-1	...	...	...	...	...	...	...	...	...
mOP-1	...	...	Gln	...	...	...	...	Leu	...
hOP-2	...	...	...	...	...	...	...	Leu	...
mOP-2	Ser	...	...	...	...	...	...	Leu	...
mOP-3	...	...	...	...	...	...	...	Leu	...
DPP	Asp	...	Ser	...	Val	...	...	Asp	...
Vg1	Glu	...	Lys	...	Val	...	...	...	Asn
Vgr-1	...	...	Gln	...	Val	...	...	...	...
CBMP-2A	Asp	...	Ser	...	Val	...	...	Asn	...
CBMP-2B	Asp	...	Ser	...	Val	...	...	Asn	...
BMP3	Asp	...	Ala	...	Ile	...	...	Ser	Glu
GDF-1	...	...	...	Glu	Val	...	...	His	Arg
60A	Asp	...	Lys	...	...	...	...	His	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Gln	...	...	...	...	...	...
		10					15		

**FIGURE 2-2**



	Trp	Ile	Ile	Ala	Pro	Glu	Gly	Tyr	Ala
hOP-1	...	...	...	...	...	...	...	...	...
mOP-1	...	Val	...	...	...	Gln	...	...	Ser
hOP-2	...	Val	...	...	...	Gln	...	...	Ser
mOP-2	...	Val	...	...	...	Gln	...	...	Ser
mOP-3	Ser	Val	...	...	...	Leu	...	...	Asp
DPP	...	...	Val	...	...	Gln	...	...	Met
Vgl	...	Val	...	...	...	Lys	...	...	...
Vgr-1	...	...	...	...	...	Pro	...	...	His
CBMP-2A	...	...	Val	...	...	Pro	...	...	Gln
CBMP-2B	...	...	Val	...	...	Lys	Ser	Phe	Asp
BMP3	...	...	...	Ser	...	Arg	...	Phe	Leu
GDF-1	...	Val	...	...	...	...	...	...	Gly
60A	...	...	...	...	...	...	...	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	Lys	...	...	...
									25
									20

**FIGURE 2-3**

hOP-1	Ala	Tyr	Tyr	Cys	Glu	Gly	Glu	Cys	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	...	...	...	...	...	Ser
mOP-2	...	...	...	...	...	...	...	...	...
mOP-3	...	...	...	...	Ala	...	...	...	Ile
DPP	...	...	...	...	His	...	Lys	...	Pro
Vgl	...	Asn	...	...	Tyr	...	...	...	Pro
Vgr-1	...	Asn	...	...	Asp	...	...	...	Ser
CBMP-2A	...	Phe	...	...	His	...	Glu	...	Pro
CBMP-2B	...	Phe	...	...	His	...	Asp	...	Pro
BMP3	...	...	...	...	Ser	...	Ala	...	Gln
GDF-1	...	Asn	...	...	Gln	...	Gln	...	...
60A	...	Phe	...	...	Ser	...	...	...	Asn
BMP5	...	Phe	...	...	Asp	...	...	...	Ser
BMP6	...	Asn	...	...	Asp	...	...	...	Ser
									35
									30

**FIGURE 2-4**

hOP-1	Phe	Pro	Leu	Asn	Ser	Tyr	Met	Asn	Ala
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	...	Asp	...	Cys	...	...	...
mOP-2	...	...	...	Asp	...	Cys	...	...	...
mOP-3	Tyr	...	...	...	...	Cys	...	...	Ser
DPP	...	...	...	Ala	Asp	His	Phe	...	Ser
Vgl	Tyr	...	...	Thr	Glu	Ile	Leu	...	Gly
Vgr-1	...	...	...	...	Ala	His	...	...	...
CBMP-2A	...	...	...	Ala	Asp	His	Leu	...	Ser
CBMP-2B	...	...	...	Ala	Asp	His	Leu	...	Ser
GDF-1	Leu	...	Val	Ala	Leu	Ser	Gly	Ser**	...
BMP3	...	...	Met	Pro	Lys	Ser	Leu	Lys	Pro
60A	...	...	...	...	Ala	His	...	...	...
BMP5	...	...	...	...	Ala	His	Met	...	...
BMP6	...	...	...	...	Ala	His	Met	...	...

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**FIGURE 2-5**

	Thr	Asn	His	Ala	Ile	Val	Gln	Thr	Leu
hOP-1	...	...	...	...	...	...	...	...	...
mOP-1	...	...	...	...	...	Leu	...	Ser	...
hOP-2	...	...	...	...	...	Leu	...	Ser	...
mOP-2	...	...	...	...	Thr	Met	...	Ala	...
mOP-3	...	...	...	...	Val	...	...	...	...
DPP	...	...	...	...	...	Leu	...	...	...
Vgl	Ser	...	...	...	...	...	...	...	...
Vgr-1	...	...	...	...	...	...	...	...	...
CBMP-2A	...	...	...	...	...	...	...	...	...
CBMP-2B	...	...	...	...	...	...	...	...	...
BMP3	Ser	...	...	...	Thr	Ile	...	Ser	Ile
GDF-1	Leu	...	...	...	Val	Leu	Arg	Ala	...
60A	...	...	...	...	...	...	...	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	...	...	...	...	...	...	...
	45					50			

**FIGURE 2-6**

hOP-1	Val	His	Phe	Ile	Asn	Pro	Glu	Thr	Val
mOP-1	...	...	...	...	...	...	Asp	...	...
hOP-2	...	His	Leu	Met	Lys	...	Asn	Ala	...
mOP-2	...	His	Leu	Met	Lys	...	Asp	Val	...
mOP-3	...	...	Leu	Met	Lys	...	Asp	Ile	Ile
DPP	...	Asn	Asn	Asn	...	...	Gly	Lys	...
Vgl	...	...	Ser	...	Glu	...	...	Asp	Ile
Vgr-1	...	...	Val	Met	...	...	...	Tyr	...
CBMP-2A	...	Asn	Ser	Val	...	Ser	...	Lys	Ile
CBMP-2B	...	Asn	Ser	Val	...	Ser	...	Ser	Ile
BMP3	...	Arg	Ala*	Gly	Val	Val	Pro	Gly	Ile
GDF-1	Met	...	Ala	Ala	Ala	...	Gly	Ala	Ala
60A	...	...	Leu	Leu	Glu	...	Lys	Lys	...
BMP5	...	...	Leu	Met	Phe	...	Asp	His	...
BMP6	...	...	Leu	Met	...	...	...	Tyr	...
		55							60

**FIGURE 2-7**

hOP-1	Pro	Lys	Pro	Cys	Cys	Ala	Pro	Thr	Gln
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	...	Ala	...	...	...	...	...	Lys
mOP-2	...	...	Ala	...	...	...	...	...	Lys
mOP-3	...	...	Val	...	...	Val	...	...	Glu
DPP	...	...	Ala	...	...	Val	...	...	...
Vgl	...	Leu	...	...	...	Val	...	...	Lys
Vgr-1	...	...	...	...	...	...	...	...	Lys
CBMP-2A	...	...	Ala	...	...	Val	...	...	Glu
CBMP-2B	...	...	Ala	...	...	Val	...	...	Glu
BMP3	...	Glu	...	...	...	Val	...	Glu	Lys
GDF-1	Asp	Leu	...	...	...	Val	...	Ala	Arg
60A	...	...	...	...	...	...	...	...	Arg
BMP5	...	...	...	...	...	...	...	...	Lys
BMP6	...	...	...	...	...	...	...	...	Lys
									70
									65

**FIGURE 2-8**

**FIGURE 2-9**

hOP-1	Asp	Asp	Ser	Ser	Asn	Val	Ile	Leu	Lys
mOP-1	...	...	...	...	...	...	...	...	...
hOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-2	...	Ser	...	Asn	...	...	...	...	Arg
mOP-3	...	Arg	Asn	Asn	...	...	...	...	Arg
DPP	Asn	...	Gln	...	Thr	...	Val	...	...
Vgl	...	Asn	Asn	Asp	...	...	Val	...	Arg
Vgr-1	...	...	Asn	...	...	...	...	...	...
CBMP-2A	...	Glu	Asn	Glu	Lys	...	Val	...	...
CBMP-2B	...	Glu	Tyr	Asp	Lys	...	Val	...	...
BMP3	...	Glu	Asn	Lys	...	...	Val	...	...
GDF-1	...	Asn	...	Asp	...	...	Val	...	Arg
60A	Leu	Asn	Asp	Glu	...	...	Asn	...	...
BMP5	...	...	...	...	...	...	...	...	...
BMP6	...	...	Asn	...	...	...	...	...	...

85

**FIGURE 2-10**



hOP-1	Lys	Tyr	Arg	Asn	Met	Val	Val	Arg
mOP-1	...	...	...	...	...	...	...	...
hOP-2	...	His	...	...	...	...	...	Lys
mOP-2	...	His	...	...	...	...	...	Lys
mOP-3	Arg	Glu	...	...	...	...	...	Gln
DPP	Asn	...	Gln	Glu	...	Thr	...	Val
Vgl	His	...	Glu	...	...	Ala	...	Asp
Vgr-1	...	...	...	...	...	...	...	...
CBMP-2A	Asn	...	Gln	Asp	...	...	...	Glu
CBMP-2B	Asn	...	Gln	Glu	...	...	...	Glu
BMP3	Val	...	Pro	...	...	Thr	...	Glu
GDF-1	Gln	...	Glu	Asp	...	...	...	Asp
60A	...	...	...	...	...	Ile	...	Lys
BMP5	...	...	...	...	...	...	...	...
BMP6	...	...	...	Trp	...	...	...	...
	90					95		

**FIGURE 2-11**

hOP-1	Ala	Cys	Gly	Cys	His
mOP-1	...	...	...	...	...
hOP-2	...	...	...	...	...
mOP-2	...	...	...	...	...
mOP-3	...	...	...	...	...
DPP	Gly	...	...	...	Arg
Vgl	Glu	...	...	...	Arg
Vgr-1	...	...	...	...	...
CBMP-2A	Gly	...	...	...	Arg
CBMP-2B	Gly	...	...	...	Arg
BMP3	Ser	...	Ala	...	Arg
GDF-1	Glu	...	...	...	Arg
60A	Ser	...	...	...	...
BMP5	Ser	...	...	...	...
BMP6	...	...	...	...	...
			100		

\*\*Between residues 56 and 57 of BMP3 is a Val residue; between residues 43 and 44 of GDF-1 lies the amino acid sequence Gly-Gly-Pro-Pro.

254GMF2054/59.50926-1

**FIGURE 2-12**



FIG. 3

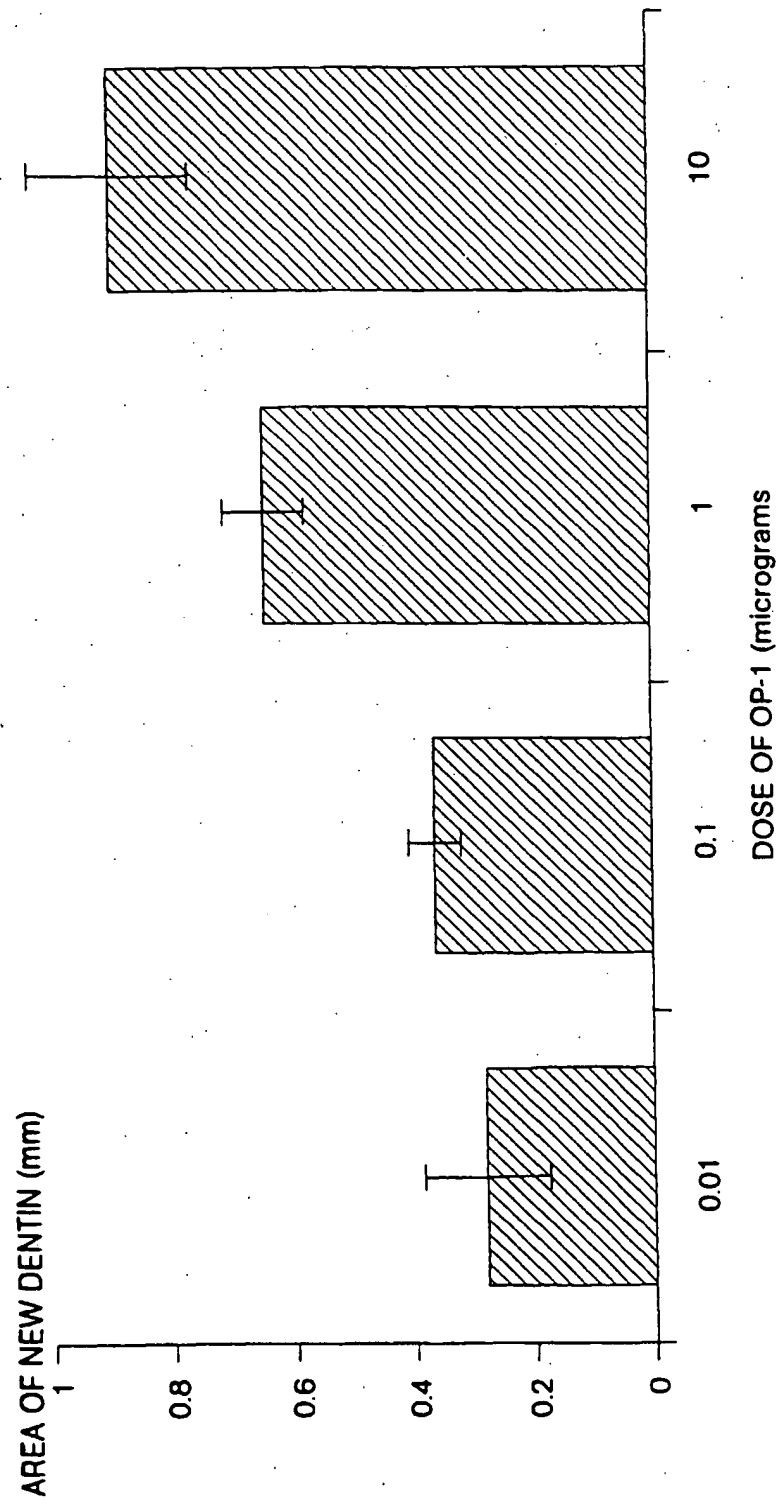


Fig. 4

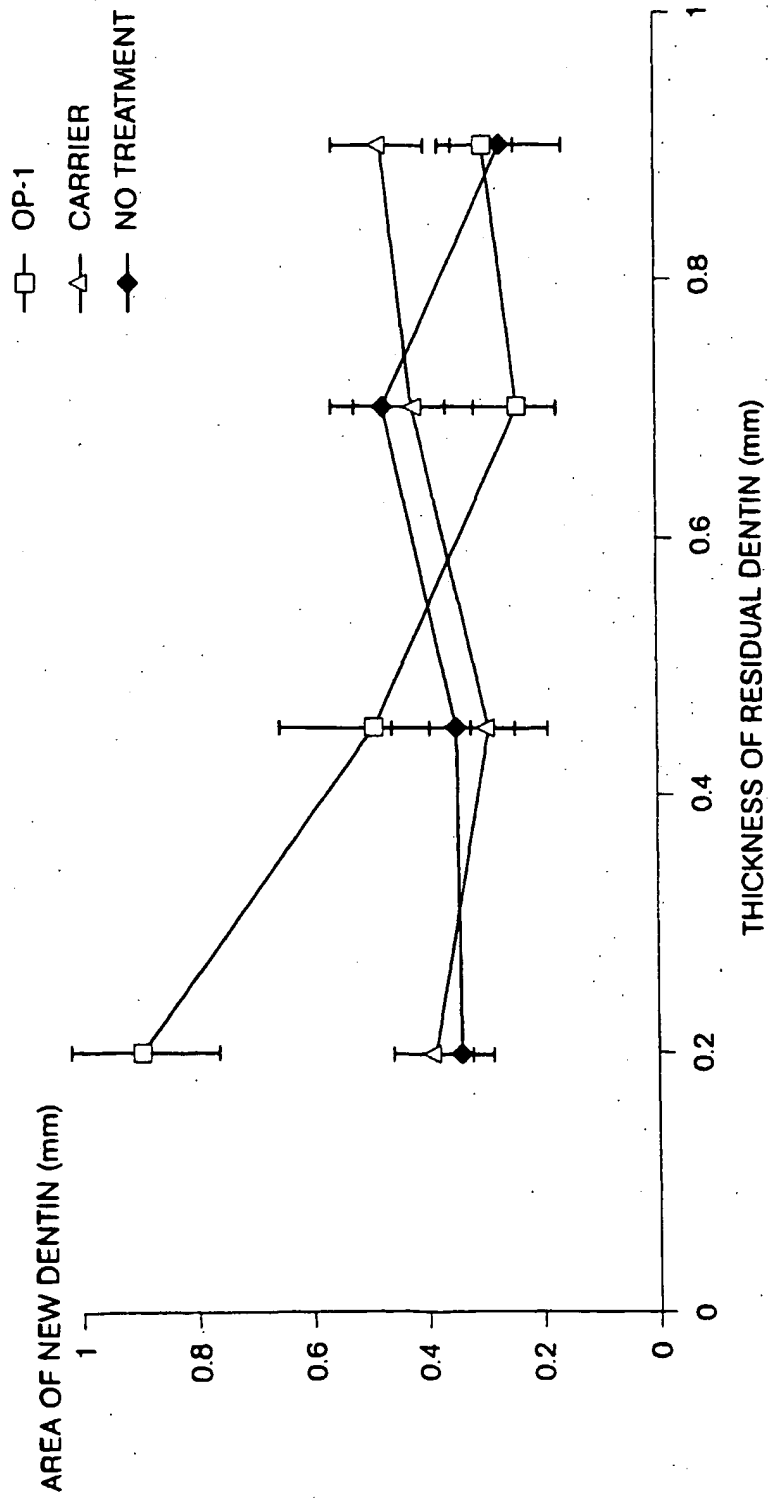


Fig. 5

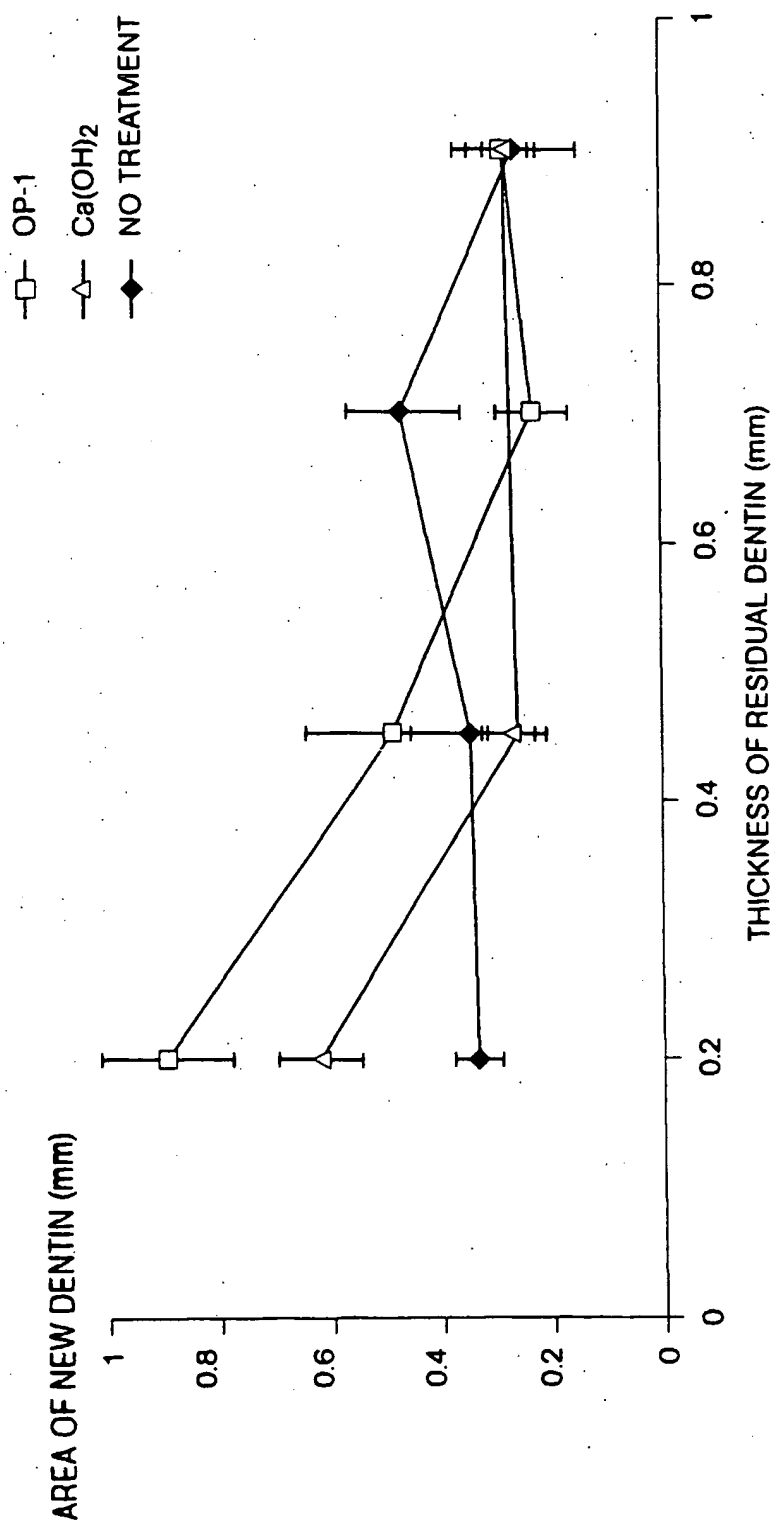


Fig. 6